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VOL. VI OCTOBER, 1911 No. 4

THE PHILIPPINE JOURNAL OF SCIENCE

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PUBLISHED BY

THE BUREAU OF SCIENCE

OF THE

GOVERNMENT OF THE PHILIPPINE ISLANDS

B. MEDICAL SCIENCES

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A COMPARATIVE STUDY OF THE AMŒBÆ IN THE MANILA WATER SUPPLY, IN THE INTESTINAL TRACT OF HEALTHY PERSONS, AND IN AMŒBIC DYSENTERY.

By ERNEST LINWOOD WALKER.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

The etiologic relation of amœbæ to endemic dysentery, while not established upon all of the postulates of Koch, is supported by clinical, pathologic, and experimental evidence and has become generally accepted; but, notwithstanding the attention these parasites have received in recent years, investigators are not agreed upon how many species are parasitic in the intestinal tract of man, whether they are obligatory parasites or whether amœbæ from water or other external sources are capable of colonizing in the human intestine, their cultivability on artificial media, and the species concerned in the production of amœbic dysentery.

The importance of these questions at issue is evident. They concern not only clinical and preventive medicine, but involve important financial considerations as well. The correct microscopic diagnosis of, and the application of early treatment to, amœbic dysentery depends upon our ability to identify the pathogenic amœba; and the requirement for radically different prophylactic measures as well as the necessity for large expenditures to distil, filter, or otherwise treat water from uncontaminated supplies in the Tropics so as to kill or remove the amœbæ which such water always contains depend upon whether or not the pathogenic amœba is an obligatory parasite.

The problem is primarily one of species, of the identity of the amœbæ that have been described by different authors in water, in cultures, in stools of healthy persons, and in amœbic dysentery. Anyone who has attempted to identify amœbæ by the descriptions in the literature will probably admit that species determination of the Amœbidæ is in a chaotic state. New species have repeatedly been established upon inconstant variations. One stage in the life-cycle of an organism has sometimes been described as one species and another stage as a second species. Authors, failing to identify the amœbæ in their possession with the incomplete descriptions in the literature, have established new species with diagnoses as indefinite as those of their predecessors.

The determination of species of protozoa, it is true, is difficult, not only on account of the minuteness and the more or less complicated life-cycles of these unicellular organisms, but also because cultural and biologic tests, that enable the bacteriologist to determine species with a certainty second only to that of the chemist in identifying the chemical elements, have a limited application to the protozoa—how limited in their application to the parasitic amœbæ will be apparent later. Protozoölogists, therefore, are forced to rely chiefly upon morphological characters to determine species. However, I believe such characters are adequate for the purpose, if a proper discrimination be made between variable and constant characters and if all the stages in the life-cycle of an organism be taken into consideration. Many of the most obvious morphological characters of the vegetative, or trophozoite, stage of amœbæ, such as shape, size, number and shape of the pseudopods, extent of the ectoplasm and contents of the entoplasm are classically variable; on the other hand the characters of the encysted stage are relatively constant and usually absolutely diagnostic: yet the descriptions in the literature of most free-living and of many of the parasitic species are based exclusively upon the trophozoite, while the more constant and distinctive characters of the cyst have been wholly neglected. It has been my experience in studying amœbæ that, if the characters of both the trophozoite and the cyst be taken into consideration, they can always be separated into species having well-defined morphologic characteristics. Therefore, it has seemed important to undertake a careful comparative study of the amœboid organisms found microscopically and culturally in the Manila water supply, in the stools of healthy person, and in amœbic dysentery for the purpose of determining the genera and species represented, their parasitism, their cultivability on artificial media, and their relation to amœbic dysentery.

REVIEW OF THE LITERATURE.

Entamœba coli (Loesch) Schaudinn, 1903, is generally considered to be the amœba first observed by Loesch in a case of dysentery at St. Petersburg in 1875. It was carefully described by Casagrandi and Barbagallo in 1897. Schaudinn distinguished it from a pathogenic species, *Entamœba histolytica*, in 1903 and found it in 50 per cent of the stools of healthy persons examined in East Prussia, in 20 per cent of the stools examined in Berlin, and in 66 per cent of the stools examined in the East Province. Craig (1905) found this species in 65 per cent of the stools examined from over 200 American soldiers stationed at San Francisco, California, and Vedder (1907) found it in 70 per cent of the stools of healthy natives in the Philippine Islands. *Entamœba coli*, as described by these authors, is round in the resting stage and opaque gray in color; the ectoplasm is scarcely differentiated from and is less refrangent than the non-vacuolated entoplasm; the nucleus is distinctly visible in the living amœba, is situated near the center of the organism, maintains its relative position in and is not deformed by the movements of the amœba, possesses a thick nuclear membrane, and contains a relatively large amount of chromatin; its movements are sluggish and it does not injest the red blood corpuscles of its host. This species reproduces by binary fission and by schizogony into 8 merozoites in the vegetative stage, and by sporogomy which is preceded by nuclear reduction and antogamy and which results in the formation of 8 sporozoite nuclei in the encysted stage. This 8-nuclear sporocyst is the resistant stage of this amœba which serves for its transmission to a new host, in the intestine of which the 8 sporozoites are supposed to be developed. Schaudinn (1903) and Craig (1908) found *Entamœba coli* non-pathogenic for kittens. Schaudinn infected himself twice with the cysts of this species without developing dysentery.

Entamœba histolytica Schaudinn, 1903, was first described by Jürgens in 1902. It was distinguished from *Entamœba coli* by Schaudinn in 1903 and was found by this author in the dysenteries of Egypt, China, and Siam, and has subsequently been identified by a number of authors in the dysenteries of different countries. This species, in contrast to *Entamœba* coli, is oval in the resting amœba and transparent or greenish in color; the ectoplasm is hyaline, viscid, distinct from and more refrangent than the vacuolated entoplasm; the nucleus, scarcely, if at all, visible in the living amœba, is eccentric in position, frequently changes its relative position in and is readily deformed by the movements of the amœba, possesses no limiting membrane, and is very poor in chromatin; its movements are active and it frequently injests the red blood corpuscles of its host. *Entamœba histolytica* reproduces by binary fission, budding, and by the formation of spores which contain chromidia derived from the nucleus and which are budded off from the surface of the amœba. These spores become surrounded by a hard, impervious membrane and constitute the resistant stage that serves for the transmission of this species to a new host. Multinuclear cysts are not developed in the life-cycle of this amœba. Schaudinn (1903) and Craig (1908) found this species to be pathogenic for kittens. Craig obtained dysentery in 50 per cent of these animals by rectal injection and in 66 per cent by feeding dysenteric stools, and lesions characteristic of amœbic dysentery and containing motile *Entamœba histolytica* were found at the necropsies.

Entamœba undulans was observed by Castellani (1905), together with entamœbæ having the characteristics of *Entamœba histolytica*, in a case of chronic dysentery followed by abscess of the liver in a European at Colombo, Ceylon. This species is characterized by a single, long, narrow pseudopod, which is shot out from the body at short intervals and quickly withdrawn, and by an undulating membrane. No observations upon its life-cycle or pathogenicity are recorded.

Paramœba hominis was found by Craig (1906) in the stools of 6 natives in the Philippine Islands in which diarrhœa, associated in 3 cases with the passage of a small amount of blood, was the only symptom. This species possesses the distinct ectoplasm, the capacity for ingesting red blood corpuscles, and the active motility of *Entamœba histolytica*, but the size, nuclear structure, and encysted stage of *Entamœba coli;* while it differs, according to Craig, from both species in its life-cycle. Besides simple division there is a development of spores within a cyst which are set free as small flagellates. The cycle is completed by the development of these flagellates into amœbæ. This organism is believed by Craig to be responsible for the diarrhœa from which the patients suffered.

Entamœba tetragina was first observed by Viereck (1907) in 2 cases of dysentery from India and designated by him as *Entamœba tetragina.* At nearly the same time Hartmann (1907) found this amœba in 11 cases of dysentery from Southwest Africa, and South America. He considered it to be distinct from *Entamœba histolytica* and gave to it the name *Entamœba africana;* but subsequently, becoming convinced of the identity of this amœba with that of Viereck, he withdrew the specific name *africana* in favor of the prior name, *tetragina* Viereck. Werne (1908) has also found this species in 9 cases of amœbic dysentery from Africa. More recently Whitmore (1911) has found it to be a common species in the dysenteries of the Philippine Islands and Indo-China. *Entamœba tetragina,* according to these authors, has the distinct, refrangent ectoplasm, the vacuolated entoplasm, the active motility, and the pathogenicity of *Entamœba histolytica;* but the distinct nucleus with a thick, nuclear membrane and rich in chromatin, characteristic of *Entamœba coli.* However, the nucleus of this species shows an arrangement of chromatin which, according to Hartmann, is characteristic and which serves to distinguish the vegetative stage from that of both *histolytica* and *coli.* In addition to the peripheral chromatin there is constantly present a small caryosome which exhibits cyclical changes and which in its most characteristic stage is made up of a minute centriol and a concentric ring of chromatin granules separated by an achromatic halo. This species, moreover, resembles *Entamœba coli* in its life-cycle. There is a sporogony in the encysted stage, but with the difference that 4 instead of 8 nuclei are developed in the sporo-cyst. *Entamœba tetragina* is considered to be a pathogenic species by all three authors. Hartmann found it pathogenic for kittens, but considers it less pathogenic than *Entamœba histolytica.* Sections of the intestine of experimentally infected animals showed a typical ulcerative dysentery.

Entamœba minuta was found by Elmassian (1909) in a case of recurrent dysentery in a European who had resided in Paraguay, South America. It possesses in the living amœba the indistinct nucleus of *Entamœba histolytica;* but the absence of a distinct ectoplasm, its sluggish movements, and its reproduction by sporogony in the encysted stage are characteristic of *Entamœba coli;* and it is distinguished from both these species by its small size, averaging from 12 to 14 microns in diameter, and by the development of 4 instead of 8 nuclei in the sporocyst. In this latter particular it corresponds with *Entamœba tetragina* Viereck. Elmassian believes that both *Entamœba minuta* and *Entamœba coli* may, under certain conditions, become pathogenic.

Entamœba nipponica was observed by Koidzumi (1909) in Japan, at first together with *Entamœba histolytica* in advanced cases, later in early cases of amœbic dysentery, and also in dysentery of bacillary origin. This species corresponds with *Entamœba histolytica* in possessing a well differentiated ectoplasm, a vacuolated entoplasm, a thin nuclear membrane, and the capacity for injesting red blood corpuscles, but with *Entamœba coli* in the richness of its nucleus in chromatin and in reproduction by schizogony in the vegetative stage; but it differs from all other species hitherto described in the arrangement of the chromatin in the forms of 3 to 8 discrete clumps on the inner surface of the nuclear membrane. Sporogony in the encysted stage was not observed. This species is considered non-pathogenic by Koidzumi.

Akashi (1911) has recently described 4 species of amœbæ parasitic in the intestinal tract of man in Japan. Two of these are considered by this author as probably identical with *Entamœba coli* and *Entamœba histolytica* respectively; one he designates as *Entamœba tetragina*, but states that he does not consider it identical with the *tetragina* species of Viereck; and the fourth is said to be a new species which the author does not designate by name. The descriptions of these species are in Japanese with a brief abstract in German. The chief point of interest in the inadequate descriptions (in German) is that reproduction takes place by "schizogony" in the encysted stage in the two latter species, with the formation of 4 nuclei in "*Entamœba tetragina*" and the number of nuclei not stated in *Entamœba* sp.

Musgrave and Clegg (1904) succeeded in producing a disease in monkeys and man having the symptoms and lesions characteristic of amœbic dysentery by introducing into the intestinal tract of the experimental animals cultures of amœbæ grown in symbiosis with pure cultures of bacteria on an alkaline agar medium consisting of beef extract 0.3 to 0.5, sodium chloride 0.3 to 0.5, agar-agar 20.0, distilled water 1000.0, and made 1 per cent alkaline to phenolphthaleïn, not only from cases of amœbic dysentery but also from lettuce and from the Manila water supply; and they were able, in certain cases at least, to recover in cultures amœbæ from the experimentally infected animals. These authors, therefore, concluded that any amœba may become pathogenic when introduced into the intestinal tract of man.

Lesage (1905) cultivated an amœba in 7 out of 30 cases of amœbic dysentery on a culture medium consisting of agar which had been washed for a week in large quantities of distilled water and on which the growth of bacteria was largely inhibited. This amœba presented many of the characters of Schaudinn's pathogenic *Entamœba histolytica* and is believed by this author to be the pathogenic species. Lesage in a subsequent paper (1907) states that leucocytic extract is a particularly suitable medium for the cultivation of the pathogenic amœbæ. This medium is prepared from the leucocytic exudate of the guinea pig. The exudate is kept on ice for 24 hours and then centrifugated. The clear, supernatant fluid is employed as the culture medium. In this medium Lesage cultivated amœbæ from cases of amœbic dysentery which were pathogenic. In 1908 Lesage cultivated a non-pathogenic amœba from the intestinal tract of man in the Tropics, which is said to represent several varieties, and which this author considers to be the non-pathogenic species of tropical countries, distinct from the non-pathogenic *Entamœba coli* of temperate climates, and to which he gave the name, *Entamœba tropicalis*.

Walker (1908) cultivated amœbæ in symbiosis with bacteria on Musgrave and Clegg's medium, from the intestinal tracts of a large number of animals. These amœbæ included one species designated as *Amœba hominis* from man. These cultures were made post-mortem from the intestines of the animals and were consequently believed to be true parasites.

Gauducheau (1908) cultivated an amœba, *Entamœba phagocytoides*, from a case of amœbic dysentery in Hanoi, Indo China, on ordinary agar, potato, and other media inoculated with *Bacillus typhosus* or other bacilli. This amœba ingests red blood corpuscles, multiplies by binary and multiple fission, and produces spirilla-like bodies in its cytoplasm.

Noc (1909) was able to cultivate an amœba three times from amœbic liver-abscesses and in five out of seven attempts from dysenteric stools in Indo China on an agar culture medium 0.5 per cent alkaline to phenolphthalein. The same species was also cultivated from the water. This amœba differs in its morphology and life-cycle from *Entamœba coli*, *Entamœba histolytica*, and *Entamœba tetragina.* Noc believes this amœba to be the cause of the amœbic dysentery and liver abscesses of that country. The cysts of this amœba, he says, are especially abundant in the surface waters of Indo China during the rainy season which corresponds with the exacerbation of endemic dysentery. However, he was unable to produce dysentery in kittens or monkeys with his cultures from water or dysenteric stools.

Werner (1908) was unable to obtain vegetative forms of either *Entamœba histolytica* or *Entamœba tetragina,* but did secure growth and cyst formation of *Amœba limax*, a common water amœba, in his cultures from dysenteric stools on Musgrave and Clegg's medium. Therefore, he is of the opinion that the amœbæ cultivated by Musgrave and Clegg (1904), Walker (1908), and others from the intestinal tract of man and other animals are cysts of similar free-living amœbæ which have been ingested with water or food, have passed unchanged through the intestine, and have found conditions favorable for development when the fæces have been placed on the culture medium.

Whitmore (1911) has also come to the conclusion that the amœbæ cultivated by him at Manila from dysenteric stools are free-living amœbæ of the *limax* type and not parasitic amœbæ.

AMŒBÆ IN THE MANILA WATER SUPPLY.

In consideration of the somewhat general belief in the Orient that the amœbæ in the water are the source of infection in amœbic dysentery, and in consequence of the cultural and experimental work of Noc (1909) Williams and Gurley (1909), and especially of Musgrave and Clegg (1904), who state that they have produced dysentery in monkeys and man with amœbæ cultivated from the Manila water supply, it has seemed desirable that a study be made of these for comparison with those found in the intestinal tract of man.

The Manila water supply comes from an uninhabited watershed of the Mariquina River and its tributaries. The water is stored in a reservoir on the watershed by a dam constructed across a narrow gorge in the Mariquina Valley at Montalban about 24 kilometers from Manila. It is conducted from this reservoir in closed water-mains to Manila, where it is distributed in branch mains and pipes to the various taps in the city. The water, therefore, is presumably free from fæcal contamination and should contain only the normal amœbic fauna of that watershed.

Samples of this water have been collected at different times from different taps in Manila for examination. In each case about 200 cubic centimeters of the water have been drawn directly from the tap into a sterile Erlenmeyer flask and about 2 cubic centimeters of ordinary nutrient bouillon added to enrich it and favor the multiplication of organisms present in the sample. The flasks, plugged with sterile cotton, were kept at room temperature and the contents examined from day to day for amœbæ. In two or three days a scum forms on the surface of the water which, if examined microscopically, will be found to consist of bacterial and protozoan growth, among the latter amœbæ are usually present. If a loop-full of this material containing amœbæ be transplanted to the surface of solidified Musgrave and Clegg's medium in a Petri dish an abundant growth of amœbæ will be obtained in a few days.

Twenty-five samples of tap water from the Manila supply have been examined microscopically, after enriching and incubating at room temperature, and by cultures made on Musgrave and Clegg's medium. As the study of the parasitic amœbæ progressed it became apparent that it was unnecessary to examine a larger number of samples, to concentrate the amœbic fauna by filtration of large quantities of the water, or to study the amœbæ from other surface waters, as had been originally intended. In 23 out of the 25 samples of water amœbæ were found microscopically and culturally. In addition to these 23 cultures of amœbæ, one culture isolated by Dr. A. W. Sellards in the Biological Laboratory of the Bureau of Science, and one isolated by Dr. E. B. Vedder at the Division Hospital of the United States Army from the Manila water supply have been studied.

Of these 25 cultures of amœbæ, twenty-four have been found to be of one and the same species (Plate I, figs. 1 and 2); one culture was a distinct species (Plate I, fig. 3). Both species appear to belong to the so-called *limax* group of water amœbæ, but because of the imperfect descriptions I have not attempted to identify them with the names in the literature, or to add to the confusion by giving them new names; nor is it necessary; it is sufficient for our purpose to determine the generic characters of these two species. This type of amœboid organism (figs. 1 to 3) is characterized by an amœboid trophozoite in which the nucleus is situated centrally in the resting organism and in which the chromatin of the nucleus is collected into a relatively large and dense karyosome that is surrounded by an achromatic halo, which possesses with rare exceptions, a contractile vacuole, and which reproduces by binary fission and by spore-formation (Walker, 1908); by an encysted stage that has a single nucleus of the same structure as that of the trophozoite, and in which no reproduction takes place; and by multiplying and encysting freely upon artificial culture media. These characters are those of the genus *Amœba* Erenberg of which *Amœba protens* Leidy is the type species.

AMŒBÆ CULTIVABLE FROM THE INTESTINAL TRACT OF MAN.

The material from which these cultures have been made has consisted of fresh stools containing amœbæ from 113 healthy persons or persons suffering from diseases other than amœbic dysentery, and from 21 cases of amœbic dysentery, and pus containing amœbæ from 2 cases of amœbic liver-abscess, obtained from the drainage tube after operation and, also, in one of the cases, post-mortem from the infected liver. This material has been obtained chiefly from the hospital of Bilibid Prison, but in part from the different hospitals in Manila. I am indebted to the physicians in charge of these hospitals and to several of my colleagues in the Bureau of Science for the opportunity and for the assistance in obtaining it.

The culture medium employed has been for the most part the standard medium of Musgrave and Clegg (1904). Several modifications of this medium have been tried, including the substitution of nutrient bouillon, peptone, dextrose, or peptone and dextrose for the beef extract, and leaving out the salt and nutrient substances altogether. The cultivable amœbæ appear to grow equally well on any of these media. The methods employed in making these cultures and in isolating pure cultures of the amœbæ are those devised by Musgrave and Clegg (1904) and by me (Walker 1908) and are sufficiently well known not to need repetition here. It has not been considered necessary in this morphologic study to isolate the pure cultures of amœbæ with pure cultures of bacteria.

Two hundred and seventy-nine cultures have been made from the stools of 113 cases of amœbiasis without symptoms of dysentery, 158 cultures from the stools in 21 cases of amœbic dysentery, and 27 cultures from the pus of 2 cases of amœbic liver-abscess, in all 464 cultures from 136 cases of amœbiasis. These cultures have been made in every case from fresh material in which living amœbæ had been demonstrated microscopically, usually in the motile condition, sometimes encysted, and often in large numbers.

Growth of amœbæ in the cultures was obtained in 6, or 5.3 per cent, of non-dysenteric cases, and in 2, or 9.2 per cent, of the cases of amœbic dysentery. All of the 27 cultures from the 2 cases of amœbic liver-abscess were negative. Growth of mastigamœbæ, flagellates, and ciliates was obtained in the cultures from several of the cases. In addition to these 8 cultures of amœba grown by myself from the intestinal tract of man, one culture isolated by Dr. E. B. Vedder from a non-dysenteric stool at the Division Hospital in Manila, one culture isolated by Dr. Marshall A. Barber from a case of diarrhœa in Kansas, one stock culture in the Biological Laboratory of the Bureau of Science from a case of amœbic dysentery, and one culture isolated by Musgrave and Clegg (1904) from a case of amœbic dysentery in Manila have been available for study.

A study of these 12 cultures has disclosed 5 well defined species (Plates II and III). Among the 8 cultures from persons showing no symptoms of dysentery there are 4 different species. Among the 4 cultures from cases of amœbic dysentery as many different species are represented. Of the latter, amœba 8 is the same species as amœbæ 1 and 20,850 from non-dysenteric stools (Plate II, fig. 4); amœba 14,965 is the same species as amœbæ 7, 7,658, and 20,998 from non-dysenteric stools (Plate II, fig. 5); and amœba 7,671 is the same species as the amœba isolated by Barber from a case of diarrhœa in Kansas (Plate II, fig. 6 and Plate III, fig. 7). Amœba 9 grown from a non-dysenteric stool and amœba "L" isolated by Musgrave and Clegg (1904) from a dysenteric stool (Plate III, fig. 8) are not duplicated among the cultures. However, amœba 9 is of the same species as the amœba cultivated from 22 out of 25 samples of water from the Manila water supply (Plate I, figs. 1 and 2). For reasons already stated no attempt has been made to identify these species with species named in the literature; it is sufficient for our present purpose, as in the case of the water amœbæ, to determine the genus to which they belong. All 5 species cultivated from both non-dysenteric and dysenteric stools possess the characters of the genus *Amœba* Ehrenberg described in the previous section (Compare figs. 1 to 3 with figs. 4 to 10).

AMŒBÆ FOUND MICROSCOPICALLY IN THE INTESTINAL TRACT OF MAN.

This part of the investigation has included a microscopic study of the living amœbæ in the fresh stools from 142 cases of amœbiasis without symptoms of dysentery, from 28 cases of amœbic dysentery, and in the pus of 2 cases of amœbic liver-abscess; of fixed and stained preparations from 21 cases of amœbiasis without symptoms of dysentery and from 16 cases of amœbic dsentery; and in sections of the intestine in 1 case of amœbic dysentery, and of the liver in 2 cases of amœbic liver-abscess. I am indebted to Dr. E. R. Stitt of the United States Naval Hospital, Cañacao, Cavite, for a section of one of the cases of amœbic liver-abscess and to Dr. V. L. Andrews of the College of Medicine and Surgery, University of the Philippines, for sections of the other case of amœbic liver-abscess and sections of the intestine from a case of amœbic dysentery.

The stools have been studied fresh in ordinary cover-glass and slide preparations. Stained preparations of the fæces and of the pus from liver abscesses have been made by spreading thin smears on cover-glasses, floating them "wet" on the surface of Zenker's fluid for 5 minutes, washing in water until the fixing fluid is removed, staining 5 minutes in aqueous alum hæmatoxylin, washing in distilled water, dehydrating in different grades of alcohol, clearing in oil origanum, and mounting in xylol balsam. I have found Zenker's fluid preferable to Schaudinn's alcohol-corrosive-sublimate mixture as a fixative for amœbæ. Aqueous alum hematoxylin has proved a more precise stain for the chromatin of amœbæ than iron hematoxylin or any other stain that I have tried. The sections from one case of amœbic live-abscess were stained with iron hematoxylin, and from the other case of liver abscess and from the dysenteric intestine with hematoxylin and eosin.

One type of amœboid organism has been found exclusively in all of this material (Plate IV, figs. 9 to 12 and Plate V, figs. 13 to 16). This differs from the *Amœba* type found in the Manila water supply and cultivable from the intestinal tract of man in certain fundamental morphological and biological characters. The trophozoite has the nucleus situated excentrically instead of centrally in the resting organism; the chromatin is arranged peripherally instead of centrally in the nucleus; there is no contractile vacuole; reproduction by sporulation does not occur; and multiplication does not take place on ordinary artificial media. The cyst contains 4 or 8 nuclei instead of a single nucleus; a reproductive process takes place in the cyst; and encystment occurs only in the body of the host (Compare figs. 1 to 8 with figs. 9 to 16). I believe, the characters of this type of amœboid organism are sufficiently distinct from the *Amœba* type to justify the establishment of the new genus, *Entamœba*, by Casagrande and Barbagallo (1897) and its adoption by Schaudinn (1903). This genus should include the parasitic amœbæ of man and also the parasitic amœbæ that have been described in certain lower animals, namely: *Entamœba ranarum* (Grassi) Dobell, 1908, in the frog, *Entamœba testudinis* Hartmann, 1910, in the turtle, *Entamœba muris* (Grassi, 1881) Wenyon, 1907, in the mouse, and *Entamœba nutalli* Castellani, 1908, in the monkey.

What, then, is the significance of the species of the genus *Amœba* cultivable from the intestinal tract of man and other animals?

These cultivable amœbæ might be considered parasitic entamœbæ that had undergone modification by their growth on artificial culture media. It seems possible that such a change in environment might modify some of the less constant morphologic characters of these organisms, such as size, shape, number of pseudopods, extent of the ectoplasm, and granulation or vacuolization of the entoplasm; but that it could cause a complete reorganization of the structure of the nucleus, develope *de novo* such a constant organelle as a contractile vacuole, or profoundly modify the life-cycle of the organism appears doubtful. Moreover, that the supposed modification resulting from cultivation on artificial media should invariably take the form of a change from the characters of the genus *Entamœba* to those of the genus *Amœba* is, to say the least, improbable.

It might be claimed that, owing to some fault in my technique, I had failed to cultivate the parasitic entamœbæ. Among my cultures are four isolated by as many different investigators, other than myself, two of which are from cases of amœbic dysentery and one of these isolated by Musgrave and Clegg (1904); all of these cultures, like my own, are of the *Amœba* type. Werner (1908) failed to cultivate entamœbæ from cases of amœbic dysentery. Dobell (1908) and Weryon (1907) also were unable to cultivate on artificial media the entamœbæ which they found in the intestines of lower animals. An examination of the descriptions and illustrations of amœboid organisms cultivated by different authors shows in every case organisms not of the *Entamœba* but of the *Amœba* type.

There still remains the possibility that the cultivable amœbæ, as well as the non-cultivable entamœbæ, are parasitic in the intestinal tract of man. Since, however, organisms of the *Amœba* type are not found in the microscopic examination of fresh or stained preparations of fæces, liver-abscess pus, or in sections of liver or intestine, it seems probably that the amœboid organisms cultivated by Musgrave and Clegg (1904), Lesage (1905, 1908), Gauduchea (1908), and Noc (1909) from the intestine and liver abscesses of man, and by myself (Walker 1908) from the intestine of man and other animals, are at most only temporary commensals in the intestinal tract, and more probably that they are, as Werner (1908) has suggested, only cysts of free-living amœbæ which have been ingested with water or food and have passed unchanged through the intestinal canal.

The entamœbæ found in the stools of healthy persons or of persons suffering from disease other than dysentery present characters that correspond in part with those of *Entamœba coli* Schaudinn and in part with those of *Entamœba nipponica* Koidzumi. In stools of such persons, in which there exists a natural diarrhœa or diarrhœa induced by a cathartic, the vegetative or trophozoite forms are commonly present. The living trophozoite is rounded in the resting condition, 7.5 to 34.5 μ in diameter, oval, ligulate or irregular when in motion, and porcelaneous and refrangent in appearance. The ectoplasm is ordinarily visible only in the pseudopods. There is no contractile vacuole and the entoplasm is normally free from all vacuolization. The nucleus is distinctly visible in the living entamœba. It occupies an eccentric position, but in the rotation of the rounded entamœba the nucleus frequently appears central in optical section. It has the form of a rather heavy ring of refrangent material, that may be of uniform or of irregular thickness, which surrounds a non-refractive nucleoplasm and within which there may or may not be present a few refractive granules. The movements of these entamœbæ are sluggish, even in fresh stools and they quickly loose all motility in cold stools.

In preparations fixed wet and stained with aqueous alum hematoxylin the trophozoite shows a deeply-staining granular cytoplasm, usually without distinction from the ectoplasm. Occasionally a narrow rim of homogeneous, more feebly-staining ectoplasm is present. The ringform nucleus is seen in optical section to consist of a nuclear membrane enclosing an archromatic nucleoplasm and a relatively large amount of chromatin. Two varieties in the distribution of the chromatin in the nucleus can be distinguished. In the first of these the chromatin appears in optical section as a continuous, segmented or granular ring of approximately uniform thickness about the inner surface of the nuclear membrane, with or without some granular chromatin scattered in the nucleoplasmic network or collected in a small karyosome. This variety corresponds with the *coli* species of Schaudinn (fig. 9). In the second variety the chromatin is collected in several discrete clumps on the inner surface of the nuclear membrane. This variety corresponds with the *nipponica* species of Koidzumi (fig. 10).

Reproduction in the trophozoite appears to take place exclusively by simple division. I have seen no evidence of a schizogony, such a described by Schaudinn in *Entamœba coli* and by Koidzumi in *Entamœba nipponica,* in either fresh or stained preparations.

In non-dysenteric stools that have become formed encysted entamœbæ make their appearance. These cysts (fig. 11) are developed, as in the case of the order Coccidiida, only in the intestinal tract of the host, and not in the evacuated fæces. If the process of encystment has already begun before the entamœbæ are passed in the stool the development of the cysts may continue outside of the host; but motile and resting entamœbæ quickly degenerate and disintegrate in cold stools. The mature cysts usually contain 8 nuclei of the same structure, but smaller and poorer in chromatin than the nuclei of the trophozoite; cysts containing more than 8 nuclei are occasionally seen. Whether such cysts represent a different species or variations in the same species, it is difficult to say. In the development of these cysts Schaudinn described a complicated series of nuclear changes, including an autogamous copulation of reduced nuclei, that preceded the formation of the 8 nuclei in the mature cyst. Such a reproductive process would constitute a sporogony comparable with that taking place in the order Coccidiida. The mature cyst would correspond with the sporocyst and the nuclei to the nuclei of the 8 sporozoites that are supposed to be developed when the cyst reaches the intestine of a new host. However, I have been unable to observe any stages in the development of the 8 nuclei in the cysts which could be interpreted otherwise than straight-forward nuclear division. Dobell (1909) has come to the same conclusion from his study of the development of the multinuclear cysts of *Entamœba ranarum*. If this interpretation of the process be correct then it must be considered either as a schizogony in which the cyst would be a schizocyst and the 8 nuclei the nuclei of the merozoites that are developed in the intestine of the new host; or possibly as a gametogony, comparable to that occurring in the order Gregarinida, in which the 8 nuclei would constitute the nuclei of gametes that are formed and which copulate in the intestine of the new host.

Entamœbæ of the *coli* and *nipponica* varieties have been observed only in the stools of healthy persons or of persons suffering from diseases other than dysentery; but it is possible that they might occasionally be found associated with another type of entamœba, about to be described, in dysenteric stools. In such cases they would represent double infections. Of the two varieties the *coli* is found much the more frequently. The *nipponica* variety may be encountered alone, alternating with the *coli* variety in different stools of the same patient, or associated with the *coli* variety in the same stool. Transitional forms between the two varieties are frequently seen, and the 8-nuclear cysts have been found associated with both the *coli* and the *nipponica* varieties of entamœba.

The entamœbæ found in the stools of cases of amœbic dysentery, in the pus of amœbic liver-abscesses, and in sections of dysenteric intestines and of amœbic liber-abscesses, present certain morphological differences from the entamœbæ found in non-dysenteric stools which appear to be fairly constant, and which in their sum serve to distinguish the entamœbæ from the two sources. The characters of the entamœbæ from dysenteric material correspond in part with those of *Entamœba histolytica* Schaudinn, in part with those of *Entamœba tetragina* Viereck and, under certain conditions, with those of *Entamœba minuta* Elmassian. The trophozoites in fresh stools in acute dysentery are usually, but not invariably, larger, more often oval in the resting condition, and more hyaline and less refrangent in appearance than the trophozoites from normal stools. The cytoplasm is uniformly hyaline in appearance with no distinction between ectoplasm and entoplasm. It contains a variable amount of granular material and often red blood corpuscles of the host as inclusions which, when collected near the center of the organism, leave a hyaline periphery that has the appearance of an extensive ectoplasm described by other authors. There is no contractile vacuole and the entoplasm is normally free from other vacuoles; it is only following changes in temperature or tonicity of the medium or in consequence of degenerative changes that vacuolization is present. The position of the nucleus is eccentric, and in the more actively motile entamœbæ in dysenteric stools the nucleus more frequently changes its relative position and is more subject to pressure that tends to deform it than the nucleus of the more sluggish entamœba in normal stools. The nucleus, in contrast to entamœbæ of the *coli* and *nipponica* varieties, is usually distinguished with difficulty in the living trophozoite. Contrary to Schaudinn I distinguish a thin but distinct nuclear membrane. This membrane encloses a homogeneous nucleoplasm which is slightly less refrangent than the surrounding cytoplasm and which contains a small amount of granular material arranged about the inner surface of the nuclear membrane and scattered in the nucleoplasm. In consequence of this thin peripheral layer and the low index of refraction of the nucleoplasm the nucleus has the appearance of an extremely pale vacuole and is distinguished with difficulty, especially in motile or vacuolated entamœbæ. These entamœbæ, in contrast to those of normal stools, exhibit an active motility in fresh stools which often persists in cold stools several hours old.

In preparations that have been fixed wet in Zenker's fluid and stained with aqueous alum hematoxylin the trophozoite in dysenteric stools usually stains feebly and shows a reticulated rather than a granular structure of the cytoplasm with no distinction between ectoplasm and entoplasm. In the stained preparations the difference in the appearance of the nucleus from the nucleus of entamœbæ in non-dysenteric stools is seen to be due to the much smaller amount of chromatin in the nucleus of the dysenteric entamœbæ. Two varieties in the distribution of the chromatin can be distinguished. In the one the chromatin is extremely scanty and is arranged as a barely perceptable layer about the inner surface of the nuclear membrane, with or without a minute karyosome or a few scattered fragments in the nuclear-network. This variety corresponds with the *histolytica* species of Schaudinn (fig. 12). In the second variety the chromatin is rather more abundant and is arranged in part as a loose granular layer, that frequently shows radial projections, about the inner surface of the nuclear membrane, and in part as a loose central karyosome which, in its most typical form, consists of a minute centriol surrounded by an achromatic halo that is bounded by a circle of chromatin granules. This variety corresponds with the *tetragina* species of Viereck and Hartmann (fig. 13).

During the active phase of amœbic dysentery only the trophozoites of these entamœbæ are present in the stools. With aqueous alum hematoxylin, which is an extremely precise stain for the chromatin of entamœbæ, only stages of binary fission have been observed. I have been unable to find any of the chromidial stages that are said by Schaudinn, Craig and Hartmann to precede the formation of spores in *Entamœba histolytica*. It seems possible that the use of iron hematoxylin or other less precise chromatin stains may have resulted in mistaking bacteria or protoplasmic granules for chromatin. I have not observed any of the later stages of the formation of the spores or the free spores in either fresh or stained preparations.

When the acute symptoms in untreated dysentery have passed and the stools of the patient are becoming normal the trophozoites become smaller, less actively motile and more rounded in the resting forms, and the chromatin becomes more abundant in the nucleus. These changes are preparatory to the development of the cysts. Such forms resemble the *minuta* species of Elmassian (fig. 14). Finally, encysted forms containing 4 nuclei appear which may persist for an indefinite period or until the patient suffers an exacerbation of the acute symptoms. Thus in amœbic dysentery 6,442 there appeared in the stools on January 30, 1911, a considerable amount of mucus and pus streaked with blood. On January 31 the stools were partly formed and partly fluid, consisting of mucus, pus, and blood and containing many amœboids, resting and encysting entamœbæ. The amœboid and resting entamœbæ were small but with the *histolytica* type of nucleus, the encysted forms were the cysts of the *tetragina* type containing 4 nuclei. The symptoms abated without treatment, the stools became normal, and the patient has had no recurrence of the disease; but the cysts containing 4 nuclei have persisted in the stools up to the present time (August 23, 1911). Still more instructive is the following chronic case, amœbic dysentery 5,748, which came down with a typical attack of amœbic dysentery on April 29, 1911, with mucus, pus, and bloody stools containing many large entamœbæ of the *histolytica* type (fig. 12). The patient was put under treatment to which he promptly responded, the symptoms abated and the stools became normal.

Cysts of the *tetragina* type then appeared in the stools. On June 1 the patient suffered a relapse with some mucus, pus, and blood in the stools and typical *Entamœba histolytica* were present in the stools. This condition has persisted intermittently in spite of treatment with ipecac until August 1 when the stools became free from mucus and blood. On August 7 they had become formed and the 4-nuclear cysts again appeared in the fæces.

The cysts containing 4 nuclei found in the stools of cases of amœbic dysentery, like the cysts containing 8 nuclei in non-dysenteric stools, are developed only in the intestinal tract of the host and not in the defecated stools. They differ from the 8-nuclear cysts in non-dysenteric stools, not alone in the different number of nuclei contained in the mature cysts, but, also, in a number of other particulars. The cysts are smaller; the nuclei are larger and contain more chromatin; the multiplication of the nuclei appears to take place earlier in the encystment of the entamœba, often even in the amœboid stage; the cyst wall, although equally impervious to stains, appears not to be so thick or well defined; and the encysting entamœba more frequently contains elongated refrangent bodies which stain deeply with iron hematoxylin, but which do not stain with the more precise aqueous alum hematoxylin, and which, therefore, are probably protoplasmic or foreign bodies rather than chromatin. (Compare fig. 11 with fig. 16.) In the development of these cysts, as in the development of the cysts from non-dysenteric stools, no indication of nuclear reduction and autogamy such as is described by Hartmann in the development of the cysts of *Entamœba tetragina,* have been observed, but only uncomplicated nuclear division (figs. 14 to 16). If these observations be correct then these cysts, like the cysts of *Entamœba coli,* would have to be considered either schizogenic or possibly gametogenic rather than sporogenic.

Entamœbæ of the *histolytica* and the *tetragina* varieties have been found only in the stools or tissues of persons suffering from or having a history of amœbic dysentery. The *histolytica* type I find, contrary to Whitmore (1911), to be the more common variety in the dysenteries of Manila. The *tetragina* variety when found, invariably has been associated with the *histolytica* and transitional forms between the two varieties are common. Moreover the *tetragina* cysts, as the protocols of the above cited cases show, have been found associated with pure *histolytica* infections.

In view of the observations that have been presented in detail in the preceding pages I am of the opinion that, although several of the varieties of entamœba hitherto described are represented, only two well defined species are found parasitic in the intestinal tract of man. One of these species includes the *coli* variety of Schaudinn and the *nipponica* variety of Koidzumi. This species is characterized by its porcelaneous and refrangent appearance, distinct nucleus and sluggish motility, in the living entamœba; by the deeply staining, granular cytoplasm, and by the relatively large amount of chromatin, which is arranged either as a heavy continuous or broken ring (*coli* variety) or as several discrete masses (*nipponica* variety) on the inner surface of the nuclear membrane, with transitions between these two varieties, in the stained entamœbæ; and especially by the development of cysts containing 8 nuclei. This species should, according to the law of priority, bear the name of *Entamœba coli* Schaudinn. It is found in the stools of healthy persons and of persons suffering from diseases other than dysentery and is, therefore, presumably non-pathogenic. The other species include the *histolytica* variety of Schaudinn, the *tetragina* variety of Viereck and Hartmann and probably the *minuta* variety of Elmassian. It is characterized by its hyaline and feebly refrangent appearance, indistinct nucleus, and active motility in the living entamœba; by the feebly-staining reticulated cytoplasm, and by the relative paucity of chromatin which is arranged either as a barely perceptible layer about the inner surface of the nuclear membrane with or without a few fragments scattered in the nuclear network (*histolytica* variety), or as a more extensive but loose, granular peripheral layer and a loose central karyosome (*tetragina* variety), with transitions between these two varieties in the stained entamœba; and especially by the development of cysts containing 4 nuclei. This species is found only in the stools, pus, or tissues of cases of amœbic dysentery, amœbic liver-abscesses, or of cases having a history of amœbic dysentery and is probably a pathogenic species. According to the law of priority this species should bear the name, *Entamœba histolytica* Schaudinn. The differences in the arrangement of the chromatin in the nuclei of the *coli* and *nipponica* varieties and of the *histolytica* and *tetragina* varieties probably represent metabolic or reproductive changes in the nuclei of the two species. This view is supported by the presence of forms showing a chromatin arrangement intermediate between these varieties of the two species. It is probably that other imperfectly described species of *Entamœba* that have been observed in the intestine of man would be found, on a more complete knowledge of their morphology and life-cycles, to belong to one or the other of these two species.

The most important practical application of these differences in morphology between *Entamœba coli* and *Entamœba histolytica* is the microscopic diagnosis of amœbic dysentery, especially in incipient or chronic cases, as a guide to treatment and prophylaxis.[1] The differentiation of

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[1] It is to be noted in this connection that the microscopic diagnosis of amœbic dysentery is not dependent upon *Entamœba hystolytica* being the primary etiologic factor in this disease, but only upon the apparently constant association of this entamœba with amœbic dysentery.

Entamœba histolytica from *Entamœba coli* must frequently be made without the help of the characteristic encysted stages. While the diagnosis in the trophozoite stage is not easy, it can be made with certainty by a protozoölogist having sufficient experience and using proper care. The examinations for this purpose should be made of perfectly fresh stools, repeated if necessary on different days, and should be made, if any uncertainty exists, of stained as well as of fresh preparations. Preparations fixed wet in Zenker's fluid and stained with aqueous alum hematoxylin should be employed for this purpose. Greater difficulty may be experienced in diagnosing chronic cases between the periods of exacerbation of the acute symptoms, in which *Entamœba histolytica,* in preparation for encystment more closely resembles in some respects *Entamœba coli;* but under these conditions a careful search will usually disclose the presence of 4-nuclear cysts. The presence of such cysts in the stool is absolutely diagnostic of *Entamœba histolytica;* but a diagnosis from the presence of 8-nuclear cysts in the stool must be made with caution, since there might exist a double infection with *Entamœba coli* and *Entamœba histolytica.*

An experimental study of the parasitism and pathogenicity of the species of the genera *Amœba* and *Entamœba,* established by this morphologic study, has been undertaken and the results of it will be presented in another paper.

SUMMARY AND CONCLUSIONS.

1. The amœboid organisms found in the Manila water supply belong to the genus *Amœba* Ehrenberg.

2. The amœboid organisms cultivable from the intestinal tract of man, both from healthy persons and from cases of amœbic dysentery, also belong to the genus *Amœba* Ehrenberg.

3. The cultivable species of the genus *Amœba* are not parasitic in the intestinal tract of man; when obtained in cultures from the intestine they probably are derived from cysts of amœbæ that have been ingested with water or food and have passed unchanged through the intestinal tract.

4. The amœboid organisms parasitic in the intestinal tract of man belong to a distinct genus, *Entamœba* Casagrandi and Barbagallo.

5. The entamœbæ are strict or obligatory parasites and are incapable of multiplication outside of the body of their host. They can not be cultivated on Musgrave and Clegg's medium.

6. One non-pathogenic species of the genus *Entamœba, Entamœba coli* Schaudinn, parasitic in the intestinal tract of man, which includes *Entamœba nipponica* Koidzumi, and which develops cysts containing 8 nuclei, is recognized.

7. One presumably pathogenic species of the genus *Entamœba, En-*

tamœba histolytica Schaudinn, which includes *Entamœba tetragina* Viereck, and which develops cysts containing 4 nuclei, is recognized.

8. A differential diagnosis of an infection with *Entamœba coli* from an infection with *Entamœba histolytica* can be made with the microscope.

9. An infection with either *Entamœba coli* or *Entamœba histolytica* must always come directly or indirectly from another infected person.

10. Water or uncooked food can transmit amœbic dysentery only when contaminated with fæcal matter from a case of amœbic dysentery.

11. The infection with *Entamœba histolytica* may persist for an indefinite period after the symptoms of amœbic dysentery have disappeared, during which time the resistant, encysted entamœbæ may be passed in large numbers in the stools and constitute an important source of infection to others. Such persons are "carriers" of amœbic dysentery, comparable to the "carriers" of typhoid fever or cholera.

12. The prophylactic measures for the prevention of amœbic dysentery are sufficiently indicated by the preceding conclusions; they are identical with those required for the prevention of other specific infectious diseases of the intestinal tract, like typhoid fever and cholera.

LITERATURE CITED.

AKASHI. Ueber die Morphologie und die Entwicklungsgechichte der Darmamoeben. *Mitt. d. Med. Gesellschaft z. Tokyo* (1911), **25**, 6.

CASACRANDI e BARBAGALLO. *Entamoeba hominis s. Amoeba coli* (Loesch). Studio biologico e clinico. *Ann. d'Igiene sper.* (1907), **5**, 1.

CASTELLANI, A. Observations on some protozoa found in human faeces. *Centralbl. f. Bakt. etc.*, Orig. (1905), **38**, 66–69.

IDEM. Note on a liver abscess of amoebic origin in a monkey. *Parasitology* (1908), **1**, 101–102.

CRAIG, C. F. Observations upon amebas infecting the human intestine, with a description of two species, *Entamoeba coli and Entamoeba dysenteriae. Am. Med.* (1905), **9**, 854, 897, 937.

IDEM. A new intestinal parasite of man. *Paramoeba hominis. Am. Journ. Med. Sci.* (1906), **132**, 214–220.

IDEM. Studies upon the amoebae in the intestine of man. *Journ. Infect. Dis.* (1908), **5**, 324–377.

DOBELL, C. C. Researches on the intestinal protozoa of frogs and toads. *Quart. Journ. Microscop. Sci.* (1909), **53**, 2,201–277.

ELMASSIAN, M. Sur une nonvelle éspèce amibienne chez l'homme, *Entamoeba minuta* n. sp. *Centralbl. f. Bakt. etc., Orig.* (1909), **52**, 3,325.

GAUDUCHEAU, A. Formation de corps spirillaires dans une culture d'amibes. *Compt. rend. Soc. biol.*, (1908), **64**, 493–494.

HARTMANN, M. Eine neue Dysenterieamoebe, *Entamoeba tetragina* Viereck, syn. *Entamoeba africana* Hartmann. Beih. 5, *Arch. f. Schiffs- u. Trop.-Hyg.* (1908), **12**, 117–127.

IDEM. Untersuchungen ueber parasitische Amoeben. I. *Entamoeba histolytica* Schaudinn. *Arch. f. Protistenk.* (1909), **18**, 2, 207–220.

IDEM. Ueber eine Darmamoeba, *Entamoeba testudinis n. sp.* Memorias do Instituto Oswaldo Cruz, (1909), **1**, 1 pl.

HARTMANN, M. und PROWAZEK, S. v. Blepharoplast, Caryosom und Centrosom. *Arch. f. Protistenk.* (1907), **10**, 306.

JURGENS. Zur Kenntnis der Darmamoeben und der Amoeben-Enteritis. *Veröffentl. a. d. Gebiete d. Militär-Sanitätswesens* (1902), **20**, 110.

KOIDZUMI, M. On a new parasitic amoeba, *Entamoeba nipponica*, found in the intestine of Japanese. *Centralbl. f. Bakt. etc., Orig.* (1909), **51**, 650.

LESAGE, A. Culture de l'amibe de la dysenterie des pays chauds. *Ann. Inst. Pasteur* (1905), **18**, 9–16.

IDEM. L'amibiase chez le chat (Dysenterie amibienne.) *Compt. rend. Soc. biol.*, (1907), **62**, 1191–1193.

IDEM. Note sur les Entamibes dans la dysenterie amibienne des pays chauds. *Bull. d. la Soc. d. Pathol. Exotique* (1908), **1**, 104–111.

LOESCH, F. Massenhafte Entwickelung von Amoeben im Dickdarm. *Arch. f. path. Anat. u. f. klin. Med.*, (Virchow), Berlin (1875), **65**, 196–211.

MUSGRAVE, W. E. and CLEGG, M. T. Amebas; their cultivation and etiological significance. *Bur. Gov. Labs.* Manila (1904), No. 18.

NOC, E. Recherches sur la dysenterie amibienne en Cochinchine. *Ann. Inst. Pasteur* (1909), **23**, 177–204.

SCHAUDINN, F. Untersuchungen ueber die Fortpflanzung einiger Rhizopoden. *Arb. a. d. Kais. Gsndhtamte.* (1903), **19**, 547–576.

VEDDER, E. B. An examination of the stools of 100 healthy individuals, with especial reference the presence of *Entamoeba coli*. *Journ. Am. Med. Asso.* (1906), **46**, 870–872.

VIERECK, H. Studien ueber die in den Tropen erworbene Dysenterie. *Arch. f. Schiffs- u. Trop.-Hyg.* (1907), **11**, Beiheft I.

WALKER, E. L. The parasitic amoebae of the intestinal tract of man and other animals. *Journ. Med. Research* (1908), **12**, 379–459.

WENYON, C. M. Observations on the Protozoa in the intestine of mice. *Arch. f. Protistenk.* (1907), Suppl. 1.

WERNER, H. Studien ueber pathogene Amoeben. *Arch. f. Schiffs- u. Trop.-Hyg.* (1908), **12**, Beiheft II.

WHITMORE, E. R. Vorläufige Bemerkungen ueber Amoeben aus Manila und Saigon. *Centralbl. f. Bakt. etc.*, Orig. (1911), **58**, 234–235.

WILLIAMS, A. W. and GURLEY, C. R. Studies on intestinal amebas and allied forms. Collected Studies from the Research Laboratory, Dept. of Health, City of New York (1908–'9), **4**, 237–246.

ILLUSTRATIONS.

(From photomicrographs by Charles Martin.)

PLATE I.

FIG. 1. Amœba 1, trophozoite, culture from the Manila water supply. × 1000.
2. Amœba 1, cysts, culture from the Manila water supply. × 1000.
3. Amœba 19, trophozoite and cyst, culture from the Manila water supply. × 1000.

PLATE II.

FIG. 4. Amœba 8, trophozoite and cysts, culture from a stool of a case of amœbic dysentery. × 1000.
5. Amœba 14,965, trophozoites, culture from a stool of a case of amœbic dysentery. × 1000.
6. Amœbæ 7,671, trophozoite, culture from a stool of a case of amœbic dysentery. × 1000.

PLATE III.

FIG. 7. Amœba 7,671, cysts, culture from a stool of a case of amœbic dysentery. × 1000.
8. Amœbæ "L" (Musgrave and Clegg), trophozoite, culture from a stool of a case of amœbic dysentery. × 1000. This species differs from Amœba 7,671 (figs. 6 and 7) chiefly in not possessing a contractile vacuole.

PLATE IV.

FIG. 9. *Entamœba coli*, trophozoite, in the stool of a healthy person. × 1000.
10. *Entamœba coli var. niponica*, trophozoite, in the stool of a healthy person. × 1000.
11. *Entamœba coli*, cyst containing 8 nuclei only 5 of which are shown in the optical section of the entamœba, in the stool of a healthy person. × 1000.
12. *Entamœba histolytica*, trophozoite containing red blood corpuscles, in the bloody and mucus stool of a case of amœbic dysentery (No. 5,748). × 1000.

PLATE V.

FIG. 13. *Entamœba histolytica var, tetragina*, trophozoite containing red blood corpuscles, in the stool of a case of acute amœbic dysentery. ×1000.
14. *Entamœba histolytica*, trophozoite in preparation for encystment, in the stool of a case of chronic amœbic dysentery. ×1000.
15. *Entamœba histolytica*, encysting entamœba showing the first division of the nucleus, in the stool of a chronic case of amœbic dysentery. ×1000.
16. *Entamœba histolytica*, cyst containing 4 nuclei, in the stool of a chronic case of amœbic dysentery. ×1000.

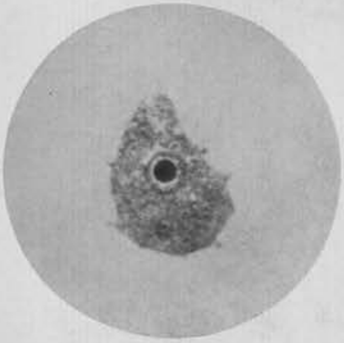

FIG. 1.

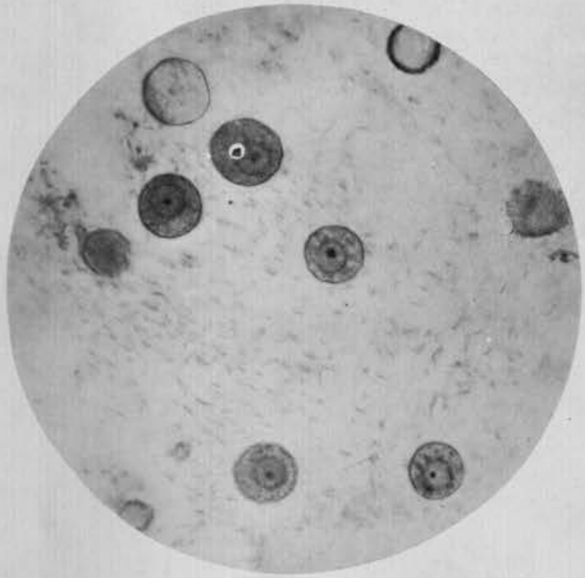

FIG. 2.

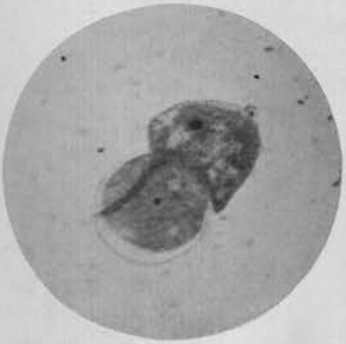

FIG. 3.

PLATE I.

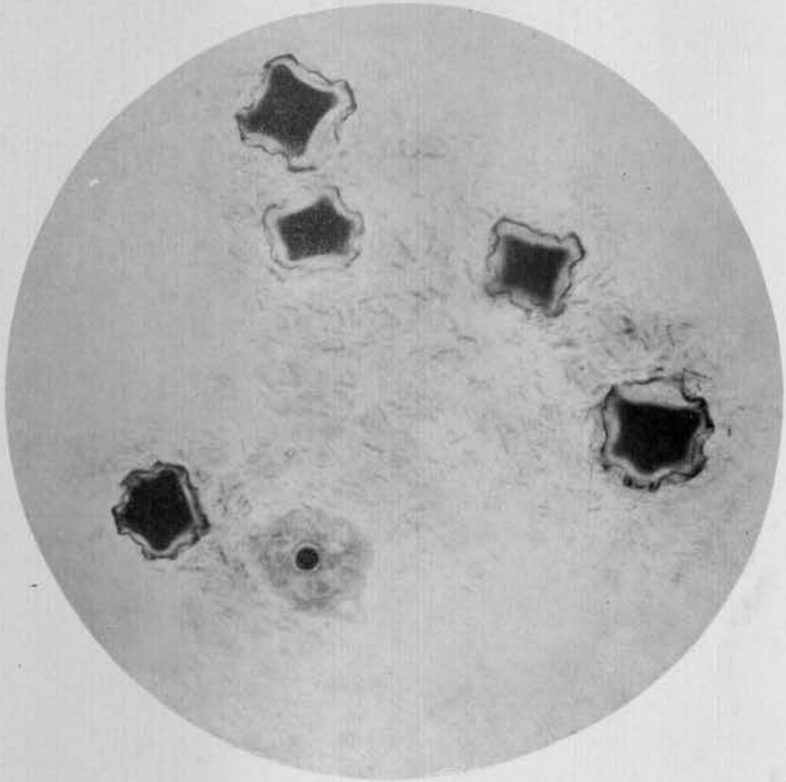

FIG. 4.

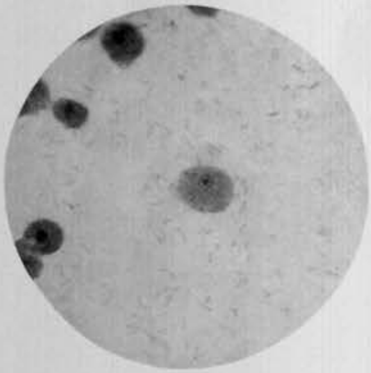

FIG. 5.

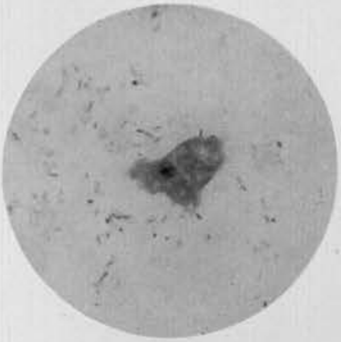

FIG. 6.

PLATE II.

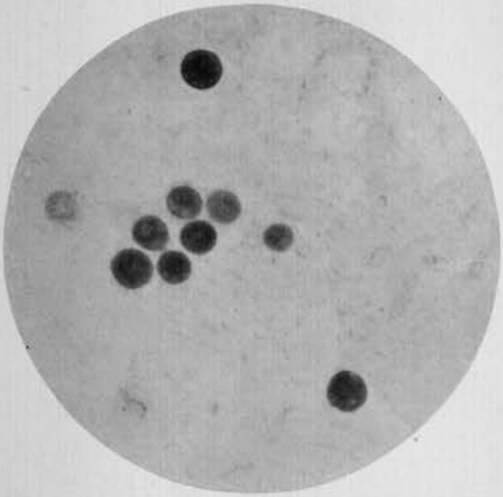

FIG. 7.

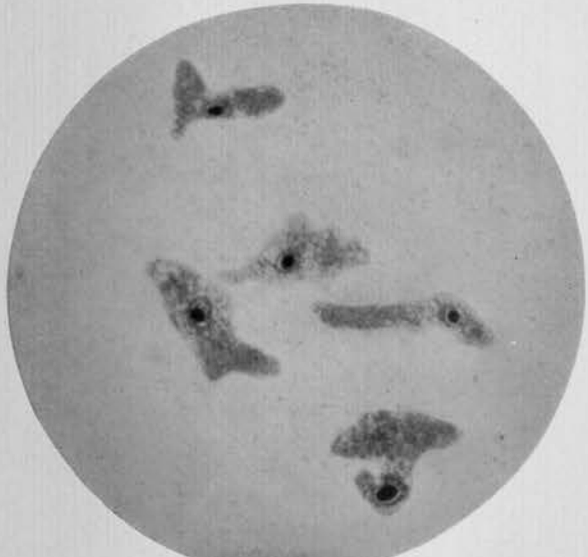

FIG. 8.

PLATE III.

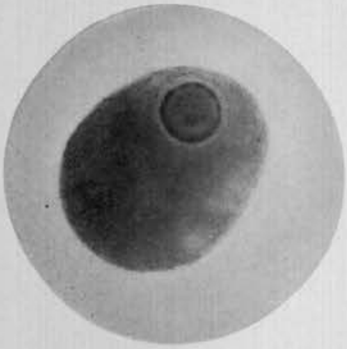

FIG 9.

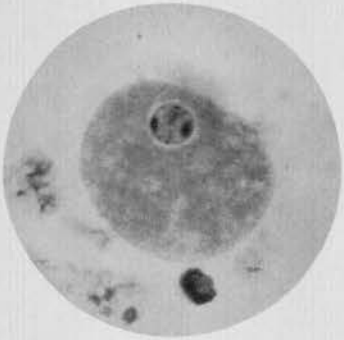

FIG. 10.

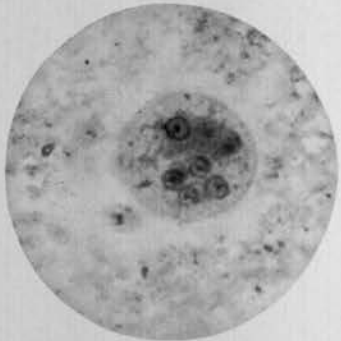

FIG. 11.

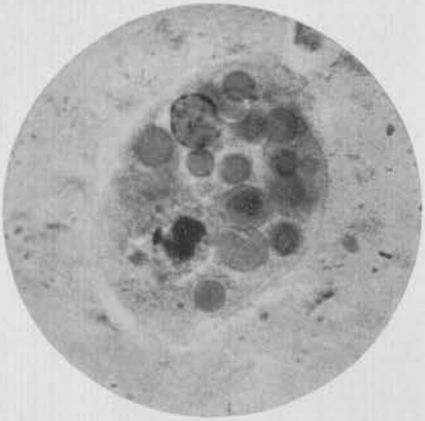

FIG. 12.

PLATE IV.

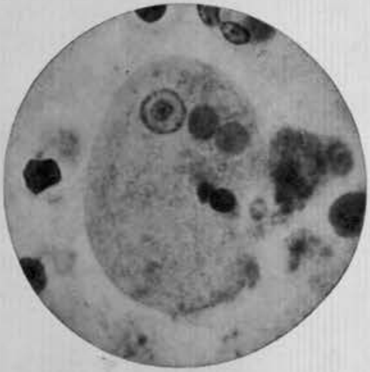

FIG. 13.

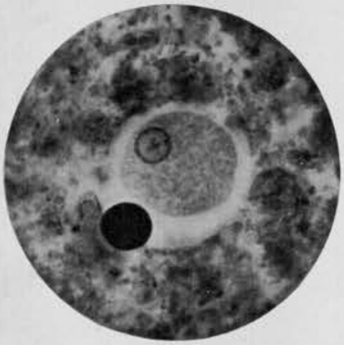

FIG. 14.

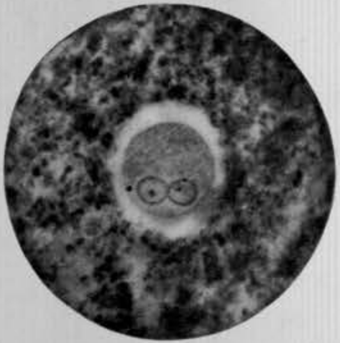

FIG. 15.

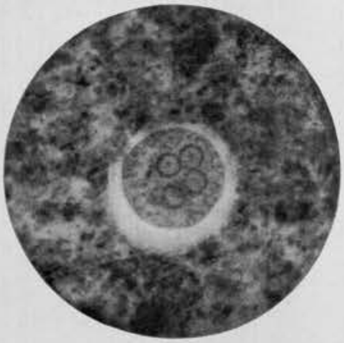

FIG. 16.

PLATE V.

IMMUNITY REACTIONS WITH AMŒBÆ.

By Andrew Watson Sellards.

(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

The aetiology and also the diagnosis of amœbic dysentery involve the question of whether non-pathogenic as well as pathogenic amœbæ may be encountered in the human intestine. In the development of the evidence bearing upon this question, two factors have arisen which are of especial interest. In the first place important morphological differences have been described for amœbæ from various sources, and secondly cultures of amœbæ have been obtained from dysenteric stools. It is maintained that definite morphological distinctions occur in the amœbæ found in the intestine with differences in clinical manifestations corresponding to the type of amœba which is present. Thus, one of these types may persist over long periods of time without the production of definite symptoms. This fact appears to constitute the principal evidence in support of the existence of a non-pathogenic species of amœba in the intestine. However, the morphological differences between this type of amœba and that occurring in active dysentery have not been accepted by certain observers, and the existence of this type as a distinct species has been questioned.

In regard to the cultivation of amœbæ, there is some uncertainty concerning the exact significance of the cultures which have been obtained from dysenteric stools. Some investigators consider that the amœbæ in these cultures are pathogenic. Others regard them as purely saprozoic forms, which are only accidentally present in the fæces, maintaining that the morphological differences are sufficient to distinguish them absolutely from the amœbæ ordinarily seen in a dysenteric stool.

In the present paper an attempt has been made to distinguish amœbæ by their biologic properties without especial reference to morphology. Immunization of animals has been carried out with various strains of amœbæ with the expectation of producing immune bodies for the comparison of the various strains. It was intended, in the first place, to determine whether any differences could be detected in the immunity reactions of cultures of amœbæ obtained from parasitic as compared with those from saprophytic sources. In the second place, the sera of dysenteric patients have been examined for changes analogous to those produced by artificial immunization with amœbæ. The number of cultures under investigation was limited to four and these were selected, without regard to morphology, from sources which represented fairly distinct conditions.

The first culture, Race A,[1] was obtained from the city water supply of Manila. This is an unfiltered river water which comes, however, from a practically uninhabited watershed. Amœbæ are constantly present in this water. Race B. was cultivated from the stools of a case of amœbic dysentery occurring in Manila. The two remaining cultures were secured from sources outside of the Tropics. Race C. was grown from hay obtained from central Illinois, a region practically free from amœbic dysentery. Race D. was cultivated in Kansas City, from the stools of a patient with slight diarrhœa.

In the preparation of these cultures for injection into animals, there are two factors which were considered to be of especial importance, namely, (1) the isolation of a single species of amœba and (2) its cultivation with a single species of bacterium.

Much of the morphologic work upon amœbæ has been carried out upon material which might contain a variety of species but in attempting a differentiation by immunity reactions it seemed especially desirable to isolate single species of amœbæ, i. e., to know that each culture consisted of the progeny of a single organism.

The most feasible method for the isolation of single cells is that devised by Barber(1) and it is readily applicable to amœbæ. In transferring single motile amœbæ from cover glasses and pipettes to nutrient media they exhibit some tendency to stick to the glassware in a manner similar to certain cells, such as leucocytes and some of the capsulated bacteria. The cysts of amœbæ, do not possess this characteristic and they can be isolated and transferred readily. Accordingly, the isolations were first attempted with the encysted stage. However, it was found that only a small proportion of the isolated cysts would multiply when transferred to agar, prepared for the cultivation of amœbæ. The substitution of a liquid, nutrient medium for the agar would reduce the manipulations to a minimum, thereby permitting the isolation of organisms in the amœboid stage without necessitating further transference in order to supply nutrient material. Normal rabbit serum was found to constitute a suitable liquid culture medium. Emulsions of motile amœbæ were prepared in serum in dilutions varying from 1 to 5 to 1 to 10 and the isolations were made immediately in hanging drops on cover slips. The entire drop was not too large to come within the field of the one-sixth or one-twelfth objective. The fluid medium was quite clear and accordingly it could be determined definitely when a single amœba was obtained. In order to supply an abundance of nutrient material the size of the droplet was increased, after isolation was effected, to a diameter of 1 or 2 millimeters. Under these conditions, multiplication took place in about one-fourth of the isolations. In the remaining three-fourth the amœbæ disintegrated, encystment rarely taking place. The hanging drop cultures were kept both at room temperature (about 25° to 30° C.) and incubator temperature (37° C.); either was found to be satisfactory. Within the first 48 hours as many as 10 to 20 organisms usually developed from the original parent cell. The drop was then transferred with a comparatively coarse pipette to agar media and the cultures were continued in the usual manner.

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[1] These cultures have been designated simply by letters, in as much as the basis for the classification of amœbæ into species is incompletely developed and the description of the various species which have been reported is often inadequate for their identification.

One race (D) had already been isolated from a single cell before it was received at the laboratory. This culture was obtained by Professor M. A. Barber of the University of Kansas from the fæces of a patient with a watery diarrhœa. Individual cysts were selected and isolated directly from the fæces. Upon transferring to nutrient media, some of these cysts developed, thus not only giving a culture consisting of a single species of amœba but also demonstrating that the cyst-like bodies seen in the fæces were true amœbæ.

The presence of bacteria in the cultures of amœbæ [2] naturally necessitates a considerable number of controls in the study of the immune bodies resulting from the injection of these mixtures. To simplify these controls, somewhat, the four different races of amœbæ were cultivated with the same species of bacterium. *B. prodigiosus* was chosen, since amœbæ grow well in conjunction with it and its chromogenic properties simplify the detection of contaminations by other bacteria. As a further control, one of the races of amœbæ was also cultivated with *Vibrio choleræ.* The separation of amœbæ from all of the accompanying bacteria except one species was found to be rather difficult. Even in the encysted stage, the amœbæ are in general rather less resistant to physical and chemical agents than the sporeforming bacteria which occur with them. In the motile stage, they are intimately mixed with bacteria, the latter clinging to the surface and also being enveloped in the substance of the amœbæ, while the spores may resist any digestive action.

The method devised by Mouton(3) was used for obtaining the amœbæ in culture with a single species of bacteria. The center of a Petri plate was inoculated with amœbæ, the desired bacterium having been inoculated previously in lines radiating from the center. As the amœbæ wandered over the plate they followed the lines of bacterial growth, gradually leaving the contaminating bacteria behind them. After several transplantations had been made in this manner, test tubes were substituted for Petri plates to avoid accidental contamination from the air. The entire surface of an agar slant was inoculated with bacteria, the inoculation of the amœbæ being made at the base of the tube. After 24 hours in the incubator with the tubes placed in the erect position, the amœbæ grew upward a distance of 3 to 5 centimeters, a sharp line of demarcation frequently appearing at the junction of the growths of the bacteria and the amœbæ. Many repetitions of this process were necessary before all of the contaminating organisms were eliminated. It was a comparatively simple matter to obtain an apparently pure culture of bacteria, but more extended observations often revealed the presence of contamination. Thus minute quantities of a culture when plated out for development of single colonies frequently showed nothing but *B. prodigiosus*, while in the course of a few weeks, other bacteria might appear in the original culture, although it had been carefully protected from contamination. It is to be noted that the culture of *prodigiosus* lost its power of producing pigment on the amœbæ agar, but this property was at once regained on the first transplantation to ordinary agar. In the isolation of amœbæ with *Vibrio choleræ*, a culture was readily obtained which showed only cholera colonies when small quantities were inoculated on ordinary agar. However, the inoculation of a large quantity of growth into a highly active anticholera serum resulted in the destruction of practically all of the cholera organisms and a spore-forming bacillus developed in the serum.

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[2] Many of the earlier observers have attempted to obtain pure cultures of amœbæ, but no successful method was established whereby development continues indefinitely in the absence of both living and dead bacteria. However, very recently, Williams(2), in a preliminary communication, reports successful cultivation in the absence of bacteria.

The stock cultures of amœbæ were always kept in test-tubes. Bacteria were added at practically every transfer and the inoculations were made in the same manner as for the purification of the cultures; that is, the entire surface was inoculated with *B. prodigiosus*, but only the base of the slant with amœbæ.

Technique of cultivation.—The majority of the various media for amœbæ depend upon the same principle, namely the reduction of the nutrient material to such a minimum that only a scanty development of bacteria can take place. Thus, there are the dilute liquid media such as the hay and straw infusions; the solid medium of Beyerinck(4) is prepared with agar which has been repeatedly extracted with water; the alkaline agar of Musgrave and Clegg(5) contains 0.03 to 0.05 per cent of beef extract and no peptone. An agar based upon the last formula was used for maintaining the stock cultures of amœbæ. The principal difference consisted in the reactions of the media, the final product when ready for inoculation being neutral to phenolphthalein. The following formula was used: Agar-agar 2.5 per cent, Liebig's beef extract 0.05 per cent, normal sodium hydroxide 2 per cent. This medium, without clarification, was sterilized at 7 kilograms pressure per square centimeter for three-fourths of an hour. After sterilization it was found that the reaction of the medium was neutral to phenolphthalein, the alkali presumably having combined with the organic material in the agar. Furthermore, under these conditions of temperature and duration of heating, the quantity of alkali which was sufficient to render the medium permanently alkaline was slightly in excess of the amount which was sufficient to prevent the agar from solidifying at room temperature. Nevertheless, although the reaction of the medium was ultimately neutral, to phenolphthalein the addition of the excess of alkali gave a better medium for the cultivation of amœbæ than was afforded by the use of agar which was just neutral or slightly alkaline before sterilization.

On agar media of the ordinary composition with an acid reaction such as are used for bacteria, no growth of amœbæ could be obtained, although some investigators have used such a medium successfully.(6) Under certain conditions, when it was desired to obtain amœba-free cultures of bacteria which were associated with amœbæ, ordinary agar was used, the first transplantations being free from amœbæ.

With the development of the methods for the cultivation of amœbæ, solid agar media have largely replaced the liquid media which were used by the earlier workers. Although straw infusions and similar fluids have been used in obtaining amœbæ for study, their isolation and propagation has been carried out chiefly on solid media. It has even been stated that, with many amœbæ, direct inoculation from solid to liquid media is difficult or impossible.(5) However it was found that some of the ordinary liquid media were very suitable for the cultivation of amœbæ, provided that these media were first diluted to such an extent that the growth of bacteria was considerably restricted. Thus, amœbæ grew well in a solution of 1 part of peptone in 1000 parts of water and fair growth occurred in ordinary nutrient bouillon when diluted to 1 part in 100, although no growth of amœbæ took place in a 0.5 per cent solution of peptone, or in bouillon of the ordinary strength. There was a fairly wide range in the concentrations of peptone which were found suitable, abundant growth taking place in dilutions varying from 1 part in 1,000 to 1 in 5,000. The amœbæ could be transferred backward and forward from the solid to the liquid media, a good growth being obtained on the first inoculation, in the same manner as with bacteria.

Occasionally, after cultivating a species of amœba for several months on the medium containing agar and beef extract, the composition was altered slightly for one or two generations by the substitutions of 0.1 per cent peptone and 0.05 per cent lactose for the beef extract. Sometimes, when the growth on agar became less abundant, a few transplantations were made in liquid media, using either diluted normal rabbit serum or a solution containing 1 gram of peptone and 0.5 gram of lactose in 1,000 cubic centimeters of water. As a general rule, the most aboundant growths of amœbæ were obtained at incubator temperature (35° C.).

One well marked exception was found to the apparent necessity of a highly diluted medium for the growth of amœbæ. All of the four cultures of amœbæ grew well in normal serum not only when it was highly diluted but also in the lower dilutions. Thus, excellent growth took place not only in dilutions of 1 to 500, but also in 1 to 2, and in 1 to 5. This ability to develop in normal serum was utilized later in testing for the production of immune sera.

Technique for immunization.—The cultures of amœbæ on agar slants were used for the inoculation of animals. Living organisms were injected in the amœboid condition, emulsions being prepared in water from 24-hour cultures. The amœbæ which were used for injection, were always grown with *B. prodigiosus*. No attempt was made to reduce the quantity of bacteria in the mixed emulsion of amœbæ and bacteria, but when care was taken to insure favorable conditions for a vigorous growth of amœbæ, the latter always predominated to a considerable extent over the bacteria.

Rabbits were used for immunization. The first two injections and sometimes the third were made intravenously and the remaining ones intraperitoneally. The injections were made at intervals of ten days to one month according to the condition of the rabbits, and the maximum period of treatment for any one animal was thirteen months. For rabbits weighing about 1.5 kilograms, one-half to one agar slant of the ordinary size, (measuring about 1.5 by 8 centimeters) upon intravenous injection was usually fatal within 12 to 18 hours. Under the same conditions, 2 slants were usually fatal upon intra-peritoneal injection. Several rabbits were used for each of the 4 cultures and varying quantities of material were injected. As a rule one-eight to one-fourth of an agar slant was used for the first injection and this was gradually increased to 6 or 8 slants. Perhaps the best result was obtained in a rabbit which at the first injection survived 1 agar slant given intravenously. Pronounced symptoms occurred; within 6 hours the rabbit became semi-comatose, the temperature rose from 39°.5 C. to 40°.1 C. and the respiratory and heart rate were too rapid to count. This rabbit received subsequent injections of amœbæ without loss of weight, or other symptoms and was eventually able to withstand 20 agar slants intraperitoneally at a single injection. Five other rabbits receiving a similar injection died within 15 hours.

The preceding technique has been observed as a routine, but other animals and other methods were also tested. Thus one series of guinea pigs was injected with cultures killed by heating to 50° C. for fifteen minutes. Whereas guinea pigs, upon intra-peritoneal injection, were able to survive only a small oese of living amœbæ as much as 1 agar slant of a killed culture could generally be used for the first injection and as many as 4 or 6 slants could be used in rabbits; however, the formation of immune bodies was not active and the subsequent injection of living amœbæ was not borne very much better than when the first injections were made with smaller quantities of the unheated material.

In one rabbit, the organisms were injected only in the encysted stage, viable cysts being given intravenously for the first two injections and the remaining injections being made intraperitoneally. This rabbit was used only in testing for agglutinins.

In as much as antibodies form readily upon the injection of vegetable cells, of animal tissues, and of unorganized protein matter, and since they occur in natural infections with protozoa, it would be expected, *a priori*, that amœbæ would also act as antigen. No attempt was made to investigate all of the immunity reactions which might be produced with amœbæ, but rather I have sought to obtain a reaction which would be suitable for distinguishing cultures from various sources. Accordingly, preliminary tests were carried out with only one of the cultures of amœbæ (Race A). Of the commoner biological reactions those for agglutinins, precipitins, cytolysins, and anaphylaxis have been considered.

Agglutination.—Agglutination tests were carried out on both the active and the encysted stages of amœbæ with serum obtained, first, from guinea pigs and rabbits injected with the motile amœbæ and second from a rabbit injected with encysted forms. In the amœboid stage, some difficulty was encountered in securing a uniform suspension of the organisms in the control preparations. The immune sera from rabbits and guinea pigs were tested in varying dilutions up to 1 in 1,000. In hanging drops, irregular clumping sometimes occurred in portions of the preparation, but differences from the control were neither constant nor well defined. In the encysted stage, uniform emulsions for agglutination were readily obtained. The serum from a rabbit which had been treated with cysts for 3 months proved to be entirely inactive. Also, the serum from rabbits treated with organisms in the amœboid stage showed no agglutinative action toward the encysted stage.[3]

Precipitins.—Examination for precipitins was somewhat complicated, partly because of the difficulty of securing a suitable extract of amœbæ and also on account of the controls rendered necessary by the possibility of the presence of bacterial precipitins.

An extract of amœbæ grown with *B. prodigiosus* was prepared by emulsifying the growth from 24-hour agar slants in the water of condensation and grinding this emulsion with sand. The fluid was collected by centrifugalization and clarified by filtration. A clear filtrate was obtained most readily by the use of a Berkefeld filter, but the precipitation reactions were more satisfactory when the extract was filtered through magnesium oxide. Serum from a rabbit which had been under treatment for five months gave a well marked precipitation in 1 to 2 and 1 to 5 dilution with the undiluted extract from the mixture of amœbæ and *B. prodigiosus.* However, this same extract also gave a precipitate with the serum from a rabbit treated with *B. prodigiosus* alone. In order to control the effect of bacterial precipitins, an extract of amœbæ growing with *V. choleræ* was tested against the serums of a rabbit immunized to amœbæ growing with *B. prodigiosus.* Only a poorly defined precipitate was obtained in 1 to 2 dilution of serum from animals which had been under treatment for from two to five months. These preliminary results, therefore, did not indicate that precipitin tests would afford a very suitable reaction for the study of amœbæ, both on account of the technical difficulties and also because of the indefinite reactions which were obtained.

Anaphylaxis.—The usual test for anaphylaxis with death of the animal requires rather more material than conveniently can be obtained from cultures of amœbæ. However, the intradermal reaction as devised by Knox Moss and Brown,(8) would be especially serviceable for work with amœbæ. Rabbits and guinea pigs were tested within two to four weeks after the first injection and also at later periods after treatment had been continued for from 1 to 6 months. The injections were made intradermally with amœbæ growing with *B. prodigiosus.* Two preparations of amœbæ were used. One consisted of an extract, filtered through magnesium oxide in the same manner as for the precipitin test. The other was merely an emulsion of a 24-hour culture in physiologic salt solution. No well defined differences were obtained between the injections in normal animals and those injected with amœbæ and *B. prodigiosus*. The bleb produced by the injection was usually absorbed within the course of an hour. After 12 to 18 hours some œdema and reddening appeared at the site of injection. This was distinctly more marked in the cases where an unfiltered extract of amœbæ was used, suppuration eventually resulting in some instances. The differences between the normal animals and the treated ones were slight and inconstant. However, the general tendency of such differences as were noted, was not in the direction of a greater reaction in the treated animals, but on the contrary these animals sometimes showed signs of less inflammation at the site of injection than the controls. The differences, however, were altogether inadequate for any satisfactory test and attention was next given to cytolysins.

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[3] However, successful agglutination of amœbæ by a non-specific serum, has been reported by Zaubitzer(7) who found that the serum of an animal when immunized to *V. choleræ*, agglutinated amœbæ growing with *V. choleræ*.

Cytolysins.—Tests for the cytolytic action of the sera were carried out microscopically. Ordinary hanging-drop preparations were made from equal parts of a suspension of amœbæ and immune serum. The suspension of amœbæ was prepared in the water of condensation from the agar culture media, in preference to normal physiologic salt solution, since the latter is hypertonic for amœbæ. A certain amount of salt could be used to advantage, perhaps, imitating the conditions which occur in surface waters, the natural habitat of some of these amœbæ, but in attempting to determine the most desirable amount of salt it was found that the amœbæ when transferred from agar to distilled water showed no definite morphologic change and remained in the amœboid stage for at least several days. The serum dilutions also were prepared with water and not with salt solution.

In testing for cytolysins, control preparations were made with normal serum, with physiologic salt solution, and with distilled water. Slight changes were noticeable immediately after preparing the mixtures. Some of the amœbæ lost their motility and assumed a spherical form, both in the preparations with immune serum and also to a lesser extent in those with normal serum and with physiologic salt solution, while in those with distilled water, no change was observed. With immune serum of a high grade cylotysis of the amœbæ could be observed microscopically and in the course of one to two hours there was a well marked diminution in the number present in the hanging drop. Sera which were sufficiently active to cause immediate cytolysis were obtained only with some difficulty and after a comparatively long period of immunization. Furthermore, none of the preparations showed complete cytolysis of all of the amœbæ, present, the differences between the normal and immune serum in this respect being only relative and not absolute. However, after 24 hours at 35° C. a definite result was readily obtained, notwithstanding the fact that rabbit serum in 1 to 2 dilution furnishes a good culture medium in which a few surviving amœbæ might develop. In the mixtures with distilled water and normal serum the amœbæ remained actively motile without any encystment before the second or third day and without any significant change in numbers. No increase in number was to be expected since the concentrations of the amœbæ in the suspension was usually greater than that which occurs in liquid culture media. In the test preparations with serum from treated animals the reaction was usually very definite. Either there were many motile amœbæ present just as with normal serum, or else there was almost a complete lysis of the amœbæ with only a few cysts and an occasional motile organism present. Occasionally, in the preparations with immune serum, many of the amœbæ took on a pink color. This was due apparently to the presence of granules of *B. prodigiosus,* although no color was visible in the suspension of bacteria surrounding the amœbæ.

The reaction in this form was then adopted for testing the behavior of the 4 races of amœbæ. The suspension of amœbæ was always prepared in the water of condensation, from an actively growing 24-hour culture in the amœboid stage. Equal parts of this suspension and the serum to be tested were mixed with a capillary pipitte according to Wright's technique and a hanging drop preparation was made from a portion of the mixture. In order to obtain constant and well defined reactions, it is necessary that the amœbæ in the culture should be abundant and should predominate over the bacteria present. The data which follow represent the conditions of the preparations after standing for eighteen to twenty-four hours at 35° C. The concentrations of serum are expressed in the final concentration after dilution with the suspension of amœbæ, for example, 1 part of undiluted serum and 1 part of the suspension is recorded as a 1 to 2 dilution of the serum.

On account of the presence of bacteria throughout the entire procedure there are several interpretations which might be considered with regard to the cause of the destruction of the amœbæ by the immune sera. Three general methods were used for detecting the effect of the bacteria. The serum of rabbits immunized to amœbæ in mixed culture with *B. prodigiosus* was tested against a culture of amœbæ growing with *B. prodigiosus* and with *V. choleræ.* Also, the serum of a rabbit immunized to *B. prodigiosus* alone was tested against a culture of amœbæ growing with *B. prodigiosus.* These controls were carried out with the culture designated A and the results of the first and second methods appear in Table I.

TABLE I.—*Production of cytolysins by injection of amœbæ in mixed culture with B. prodigiosus.*

Serum of rabbit immune to—	Agglutination of *B. prodigiosus*—				Lysis of amœbæ in cultivation with—			
	In pure culture at dilution of—		In mixed culture with amœbæ at dilution of—		*B. prodigiosus* at dilution of—		*V. choleræ* at dilution of—	
	Experiment I.	Experiment II.	Experiment I.	Experiment II.	Experiment I.	Experiment II.	Experiment I.	Experiment II.
B. prodigiosus	1-50	1-100	1-50	1-50	None. 1-2	None. 1-2	None. 1-2	None. 1-2
Amœbæ and *B. prodigiosus*	1-5	1-5	1-10	1-5	1-5	1-10	1-5	1-5
Control, normal rabbit serum	Slight. 1-2	None. 1-2	None. 1-2	None. 1-2	None. 1-2	None. 1-2	None. 1-2	None. 1-2

From these data it appears that the lysis of the amœbæ was due to the immune bodies formed in response to the injection of the amœbæ, and not to the accompanying bacteria, since the amœbæ growing with cholera were acted upon by the serum produced by the injection of amœbæ and *B. prodigiosus,* whereas the serum produced by the injection of *B. prodigiosus* alone was inactive for amœbæ. In the comparison of the different races of amœbæ it will be seen that there is some additional evidence in support of the preceding conclusions.

The activity of the serum is of extremely low grade even for protozoan material. The highest serum which was obtained at any time was that from a rabbit which had survived on the first injection 1 agar slant of amœbæ. The same dose was fatal for 5 other rabbits. Five months after the first injection, when the rabbit was able to withstand 4 agar plants intraperitoneally at one injection, the serum was found to be active in 1 to 50 dilution. This degree of activity persisted for only 2 days and then fell to 1 to 10; subsequent injections failed to increase it. Apparently, the repeated injections of amœbæ instead of producing highly active sera only caused the low grade of activity to persist over longer periods. In considering the degree of activity which was obtained, it is to be noted that the amounts of material injected were rather lower than are ordinarily used in working with animal cells, such as red blood corpuscles. The quantities used were probably not as great as are usually employed for many of the ordinary bacteria such as *V. choleræ* or *B. typhosus.* Two factors were concerned in producing this limitation, namely the relatively small quantity of amœbæ which can be obtained from the growth on agar and the inability of animals to withstand the injections, emaciation and death resulting from an over dosage.

Character of the immune bodies.—A few tests were made to determine whether the destruction of the amœbæ was accomplished by the combined action of amboceptor and complement in a manner analogous to the action of hæmolysins and bacteriolysins. The effect of heating is shown by the results in Table II. The serum of a rabbit immunized to amœbæ (race A) and *B. prodigiosus* was tested against the same mixture of amœbæ and bacteria. One part of serum was diluted with 1.5 parts of water for the purpose of heating at 70° and 78°.

TABLE II.—*Showing effect of heat on immune serum.*

Lysis produced by heated immune serum.	Dilution of immune serum.	Temperature and duration of heating.					Unheated immune serum.
		60°, one-half hour.	60°, 1 hour.	60°, 3 hours.	70°, one-half hour.	78°, one-half hour.	
With complement added:							
Normal rabbit serum—							
1 to 10	1-2	Active.	Active.	Active.			
	1-5	Active.	Active.	Active.	Active.	Active.	
	1-10	None.	None.	None.	None.	Slight.	
1 to 5	1-2	Active.	Active.	Active.			
	1-5	Active.	Active.	Active.	Active.	Active.	
	1-10	None.	None.	Slight.	None.	Slight.	
Without complement	1-2	Active.	Active.	Active.			Active.
	1-5	Active.	Active.	Active.	Active.	Active.	Active.
	1-10	None.	None.	Slight.	Slight.	Slight.	Slight.

This activity of the serum after exposure to relatively high degrees of temperature seems at first a little unexpected. However, related phenomena have been observed with other protozoa and even with bacteria. Thus Rössle (9) found that an immune serum produced by the injection of paramœcia was not inactivated after exposure for 30 minutes to a temperature of 70° C. However Rössle does not describe this serum as a true cytolysin and, furthermore, it was thermostable only when the injections were made with killed and not with living paramœcia. Laveran and Mesnil (10) found that an immune serum for trypanosomata was only partially inactivated after exposure to a temperature of 64° C for one-half to three-fourths of an hour. Later, Hamilton (11) reported a thermostabile bacteriolysin for an organism designated as the "Ruediger bacillus."

However, these results with amœbæ do not prove that the immune body is thermostable, unless normal heated serum also shows no activity. Accordingly, the effect of heat was tested both on normal serum and on the serum of a rabbit which had been immunized. This rabbit had formerly shown some immunity, its serum being active in 1 to 5 dilution. At the time of this test no injections of amœbæ had been given for 2 months and as seen in Table III, the unheated serum was inactive.

TABLE III.—*Lysis of amœbæ by heated sera.*

	Dilution.	Serum, formerly immune to amœbæ.	Control, normal rabbit.
Control, unheated	1-2	None.	None.
	1-5	None.	None.
	1-10	None.	None.
Serum heated for one hour at—			
60° C.	1-2	Active.	Active.
	1-5	None.	None.
	1-10	None.	None.
70° C.	1-5	Active.	None.
	1-10	None.	None.
78° C.	1-5	Active.	None.
	1-10	None.	None.

This result shows definite activity of the normal serum when heated at 60° and of the immune serum at 60°, 70°, and 78° C.

An additional test was carried out to determine whether this apparent difference between the normal and immune sera at higher temperatures is a specific property of anti-amœba sera or whether it is common to other immune sera. The sera of a rabbit immune to *V. choleræ* was used and a second rabbit formerly immune to amœbæ also tested in addition to the one used in the preliminary experiment. The results are given in Table IV.

TABLE IV.—*Lysis of amœbæ by heated sera.*[4]

	Dilution.	Serum formerly immune to amœbæ.		Serum immune to *V. choleræ*.	Controls, normal rabbit sera.	
		A.	B.		A.	B.
Control, unheated	1-2	None.	None.	None.	None.	None.
	1-5	None.	None.	None.	None.	None.
	1-10	None.	None.	None.	None.	None.
Serum heated for one hour at—						
60° C.	1-2	Active.	None.	Active.	Active.	Active.
	1-5	None.	None.	None.	None.	None.
	1-10	None.	None.	None.	None.	None.
70° C.	1-5	Active.	Active.	None.	None.	None.
	1-10	None.	None.	None.	None.	None.
78° C.	1-5	Active.	Active.	None.	None.	None.
	1-10	None.	None.	None.	None.	None.

[4] The word lysis has been retained in speaking of the action of the serum on the amœbæ although the process, at least in the case of the heated normal serum, differs from that of the ordinary specific lysins. With an immune serum some solution takes place within the first few hours and although a period of eighteen or twenty-four hours may be required to complete the process, yet it is accomplished by a direct action on the amœbæ and it is not a secondary effect of the action of the antibacterial serum on the accompanying bacteria. Apparently the

In one instance in Table IV it will be noted that lysis of the amœbæ failed to occur in the 1 to 2 dilution of serum heated at 60° C. An emulsion was used which was unusually rich in amœbæ and, although the differences were well marked after the preparations had stood for 18 hours, yet the amœbæ did not entirely disappear until after 24 hours. These results might be taken as indicating that exposure to a temperature of 60° C. for one hour represents about the minimum time which would be effective in producing this change in the serum. Perhaps a more moderate heating might have inactivated the immune serum without producing this change which renders normal serum active. Although it is definitely shown that the immune serum is active after heating yet the controls with normal serum show that it does not necessarily follow that the immune bodies are thermostable or that they do not consist of amboceptor and complement. No attempt was made to work out the mechanism of this action of the heated normal serum. It is possible, of course, that it does not act directly upon the amœbæ, but affects them secondarily through a primary action on the bacteria; for example, the bacterial products formed in heated serum may be different from those in unheated serum. *Apriori,* however, the simplest explanation is that of a direct action upon the amœbæ. There is one instance in which heated normal serum can be shown to have a definite action on animal cells:

This phenomenon, which may be somewhat analogous to the reaction with amœbæ, can be demonstrated with red blood corpuscles[5] when heated normal serum develops the property of causing marked rouleaux formation of red corpuscles suspended in salt solution. The degree of heating which is required and the activity of the serum correspond rather closely to the conditions pertaining to amœbæ; thus, serum after exposure to 60° C. for 1 hour is effective in producing rouleaux in a final dilution of 1 to 4, but not in 1 to 6; normal serum after the same treatment is active toward amœbæ in 1 to 2 but not in 1 to 5 dilution. It may be noted that this action of heated sera in causing rouleaux formation is apparently only an increase of a normal characteristic of unheated sera. Similarly, it can be shown that although amœbæ grow well in a 1 to 2 dilution of normal rabbit serum, yet they are destroyed in undiluted normal serum, or in a 1 to 2 dilution of heated normal serum.

BIOLOGIC RELATIONSHIP BETWEEN THE DIFFERENT CULTURES OF AMŒBÆ.

The serum reactions with the 2 cultures of amœbæ obtained from saprophytic sources were compared with 2 cultures obtained from the intestinal tract to determine their indentity or non-indentity as tested by biologic methods. Rabbits were immunized in a similar manner to

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term lysin is seldom applied to immunity reactions with protozoa. Rössle(8) observed that instead of lytic properties, cytotoxic sera often possess a paralyzing action; the examples cited are the spermotoxic sera, and the anti-sera against epithelium and against paramœcia.

[5] *Bull. Johns Hopkins Hosp.* (1908), 19, 271.

the 4 races of amœbæ, all of which were growing in conjunction with *B. prodigiosus*. Complete tests for the comparison of the 4 races of amœbæ were carried out simultaneously, using sera of a low grade but sufficiently active to give well-defined results.

The data for these tests are shown in Tables V and VI.

TABLE V.—*Biological relationship of the various cultures of amœbæ.*

[First determination.]

Serum.		Lysis of amœbae.			
Immune to—	Dilution.	Race A.	Race B.	Race C.	Race D.
Race:					
A	1-2	Active.	None.	None.	Slight.
	1-5	Active.	None.	None.	None.
	1-10	Active.	None.	None.	None.
B	1-2	None.	Active.	None.	None.
	1-5	None.	Active.	None.	None.
	1-10	None.	Slight.	None.	None.
C	1-2	None.	None.	Active.	None.
	1-5	None.	None.	Active.	None.
	1-10	None.	None.	Active.	None.
D	1-2	None.	None.	None.	Active.
	1-5	None.	None.	None.	None.
	1-10	None.	None.	None.	None.
Normal rabbit serum	1-2	None.	None.	None.	None.
	1-5	None.	None.	None.	None.
	1-10	None.	None.	None.	None.
Control with water	----------	None.	None.	None.	None.

TABLE VI.—*Biological relationship of the various cultures of amœbæ.*

[Second determination.]

Serum.		Lysis of amœbæ.			
Immune to—	Dilution.	Race A.	Race B.	Race C.	Race D.
Race:					
A	1-2	Active.	None.	None.	None.
	1-5	Active.	None.	None.	None.
B	1-2	None.	Active.	None.	None.
	1-5	None.	None.	None.	None.
C	1-2	None.	None.	Active.	None.
	1-5	None.	None.	Active.	None.
D	1-2	None.	None.	None.	Active.
	1-5	None.	None.	None.	Slight.
Normal rabbit serum	1-2 1-5	None.	None.	None.	None.
Control with water	----------	None.	None.	None.	None.

It is clear from these tables that the 4 races of amœbæ are, biologically at least, distinct species, since the sera produced by them show a definite specificity. Here again it seems a little unusual that the first four races selected should have reacted in absolutely different manners, not even group reactions being present. In Table V, the serum of race A apparently showed some activity against race D. Here it was thought that perhaps only a relative difference existed between the activity of serum A for amœbæ A and D, but subsequent tests did not confirm this view. In Table VI the difference is absolute and this result was confirmed by two subsequent tests. This behavior of the sera corresponds with the results obtained by Rössle(9) who found that the sera produced by paramœcia are also specific.

The specificity of the sera for the corresponding cultures affords additional evidence that the injections of the amœbæ were responsible for the activity of the sera, since it was possible for the amœbæ to vary in each culture, but the bacterial species, *B. prodigiosus*, was common to all. If any activity of the antibacterial serum against the *B. prodigiosus*, in the symbiotic culture of amœbæ and bacteria could have affected the growth of the amœbæ, then this effect should have shown itself in all four cultures.

A note on the morphology of these cultures is of interest in view of the biologic differences which were found. Dr. E. L. Walker of the biological laboratory, Bureau of Science, has very kindly examined these cultures and describes them as being readily distinguishable from each other on a morphologic basis, however all correspond to the *limax* type.

REACTIONS WITH SERA FROM DYSENTERIC PATIENTS.

The interpretation of these biologic differences requires some caution. Many possibilities present themselves in view of the failure of these amœbæ to fall into groups according to the source from which they were obtained. Thus any of these amœbæ may be pathogenic or any may be harmless. In the case of the cultures obtained from dysenteric fæces, it is possible that the pathogenic species may fail to grow on artificial media and the organism which does develop may be merely an accompanying saprozoite.

Accordingly, in an attempt to determine whether these amœbæ bear any ætiologic relationship to amœbic dysentery, these four races were tested against the sera of amœbic dysentery patients. In considering the possibility of the formation of immune bodies during the course of the disease it is of some interest to note whether there is any indication of the absorption of toxic products from the intestine which might serve as antigen. In the first place, it is note-worthy that there is some opportunity for such absorption since the amœbæ penetrate deeply, in large numbers, into the submocosa of the intestine. Second, changes in the formed elements of the blood are known to occur, a leucocytosis sometimes being present involving not only the polymorphonuclear neutrophiles but in some instances the mononuclear neutrophiles and the eosinophiles as well. Also, in severe infection some fever is often present. Perhaps the most favorable cases for the absorption of antigen and the production of immune bodies would be those in which infection of the liver with abscess formation has occurred. However, one would not expect to find very active sera in view of the low grade of immunity in protozoan infections generally and because of the comparatively slight response of animals to the injections of amœbæ.

Samples of sera were taken in the stage of active dysentery, during convalescence, and after recovery, the majority of cases having been treated with ipecac. The diagnosis of the cases was based upon the presence of a dysentery resembling the usual course of the amœbic type together with the finding of amœbæ miscroscopically in the stools. No cases were available in which spontaneous recovery took place, although some had experienced repeated relapses alternating with periods of several months in which no symptoms were present.

The sera from these cases were tested against the 4 races of amœbæ. As a routine, the serum was used in dilutions of 1 to 2, 1 to 5, 1 to 10, 1 to 20, 1 to 50, and 1 to 100, the final readings being made after 18 to 24 hours. In the first case which was tested, the sample of serum was taken 4 days after the subsidence of an acute attack of dysentery. A definite reaction took place at 1 to 2 dilution with Race A, the amœbæ obtained from the city water supply. The preparations in the other dilutions and with all of the other amœbæ behaved like the controls prepared with normal serum. In a repetition of this test 4 days later, no reaction was obtained in any dilution with any of the 4 races of amœbæ. The second patient who was examined had exhibited symptoms of chronic dysentery for many months. The serum from this case reacted only in 1 to 2 dilution with Race B, the amœbæ cultivated from the stools of a dysenteric patient. All of the other preparations showed no lysis. There was no opportunity for confirming this result. The remaining cases that were examined consisted of 5 acute cases, 8 chronic ones, and 3 with liver abscess. The acute cases were examined while the symptoms were active and also within two to four weeks after recovery. None of these reacted with any of the races of amœbæ that were tested. The 8 chronic cases were examined during a period of relapse and 5 of these were also tested at a later period when they were free from symptoms. All, however, reacted like the normal control sera, the amœbæ remaining numerous and active in the 1 to 2 preparations and in all of the higher dilutions. Of the 3 cases of amœbic abscess of the liver, one was tested 4 days before exitus and the other two were tested at operation and after recovery. The sera of these cases produced no lysis with any of the cultures of amœbæ, and these cultures failed to agglutinate with the sera from the human cases.

From the behavior of these tests, it seems probable that the reactions obtained in the first 2 cases were merely accidental. The reaction resulted in one case with amœbæ secured from a well-protected river-water, and in the other with amœbæ cultivated from a dysenteric stool. In both instances, the tests were carried out at a time when the cultures were growing only moderately well. Both reactions were obtained in 1 to 2 dilution and it is not unlikely that the absence of amœbæ is to be accounted for by the excessive growth of bacteria.

In as much as these tests failed to show any evidence of immunity, it seemed desirable to carry out some of the reactions with amœbæ known to be pathogenic, and to this end an attempt was made to utilize dysenteric stools in which the amœbæ were very numerous. The serum reaction as used with cultures of amœbæ, requires some modification in applying it to a dysenteric stool, since the amœbæ disintegrate within a few hours in the stool itself, or in any ordinary culture medium. Accordingly, mixtures of stools containing numerous amœbæ and the sera from dysenteric patients were made in the same manner as for cultures of amœbæ to determine whether any changes would take place within a few hours, before the control preparations deteriorated.

Two patients were selected in which the stools consisted largely of blood and mucus, practically free from fæcal matter and in which the amœbæ were numerous in every field. Equal parts of these stools were mixed, first with the undiluted sera from the corresponding patients; second, with the serum from a case with acute symptoms of dysentery; and third, with serum from a patient who about three weeks previously had recovered from an acute attack of dysentery. Control preparations were made from normal serum. The amœbæ remained alive and active for a period of 2 hours. At the end of 3 hours the motility was much diminished in all preparations and the amœbæ were disintegrating in the preparations with the sera of dysenteric cases and in the control preparations as well. Further work upon the aetiology and diagnosis of amœbic dysentery by immunity reactions did not appear to be feasible on account of the lack of cultures known to be pathogenic.

SUMMARY.

The injection of cultures of amœbæ into rabbits resulted in the production of serum which was cytolytic for amœbæ.

This serum possessed only a low grade of activity. No inactivation resulted after exposure to a temperature of 60° C. for periods varying from thirty minutes to three hours or 70° C. for thirty minutes. However, the anti-bodies of the serum were not proved to be thermo-stable, since normal serum became active when heated for one hour at 60° C.

Of the sera produced by 4 cultures of amœbæ obtained from parasitic and from saprophytic sources each serum was cytolytic for the corresponding culture, but not for the other three. This specificity indicates that these amœbæ are biologically distinct. Subsequent examination showed that these amœbæ are also distinguishable from each other upon a morphologic basis.

Examination of the sera of amœbic dysentery patients failed to demonstrate any production of immune bodies for amœbæ during the course of the disease. However, the method which was employed was not satisfactory when applied directly to amœbæ occurring in bloody mucus stools. Consequently, the results do not represent any reaction upon amœbæ which are known to be pathogenic. Tests upon cultures of amœbæ did not give any definite evidence of the presence of either cytolisins or agglutinins in the patient's serum.

Unfortunately these results do not lead to any definite conclusion but merely indicate that either immune bodies were not produced or that the cultures under consideration do not bear any ætiologic relationship to amœbic dysentery. Certainly there is no evidence of pathogenicity; however, on the other hand, there is nothing but negative evidence indicating their harmlessness.

REFERENCES.

(1) Barber. *Univ. Kansas Sci. Bull.* (1907), **4**, 3 and *Journ. Infect. Dis.* (1908), **5**, 379.
(2) Williams. *Proc. Soc. Exp. Biol. Med.* (1911), **8**, 56.
(3) Mouton. *Ann. Inst. Pasteur* (1902), **16**, 457.
(4) Beyerinck. *Centralbl. f. Bakt. etc. Orig.* (1896), **19**, 257.
(5) Musgrave and Clegg. *Bur. Gov. Labs.* Manila (1904), No. 18.
(6) Williams and Gurley. Collected Studies, Research Lab. Dept. Health, City of New York, (1908–9), **4**, 237.
(7) Zaubitzer. *Arch. f. Hyg.* (1901), **40**, 103.
(8) Knox, Moss, and Brown. *Journ. Exp. Med.* (1910), **12**, 562.
(9) Rössle. *Arch. f. Hyg.* (1905), **54**, 1.
(10) Laveran and Mesnil. *Ann. Inst. Pasteur* (1901), **15**, 673.
(11) Hamilton. *Journ. Inf. Dis.* (1908), **5**, 570.

TYPHOID FEVER IN THE PHILIPPINE ISLANDS.

By WESTON P. CHAMBERLAIN.[1]

(From the United States Army Board for the Study of Tropical Disease as they Exist in the Philippine Islands.)

PART I. PREVALENCE AND DISTRIBUTION OF TYPHOID FEVER IN THE PHILIPPINES.
PART II. LABORATORY INVESTIGATIONS OF TYPHOID FEVER IN THE PHILIPPINES.
PART III. CLINICAL ASPECTS OF TYPHOID FEVER IN THE PHILIPPINES.
PART IV. A STUDY OF RECENT TYPHOID EPIDEMICS IN THE PHILIPPINES.
PART V. CONCLUSIONS.

PART I. PREVALENCE AND DISTRIBUTION OF TYPHOID FEVER IN THE PHILIPPINES.

INTRODUCTION.

From December 1, 1908, to date of this writing (April 30, 1911) the Board for the Study of Tropical Diseases has been making for the Philippines Division the routine blood cultures and Widal examinations on the military patients suspected of having typhoid fever and, also, making for convalescents the cultural examinations of the stools and urine which are required before a soldier who has had typhoid is allowed to return to duty. For nearly all cases in which we obtained positive laboratory findings a clinical history has been furnished to the Board. From this laboratory and clinical material we have been able to learn much of interest as regards typhoid in the Philippine Islands.

The greater part of our material for blood culture comes from a considerable distance, the time consumed *en route* varying from a day to several weeks. The same applies to the stool and urine specimens, and, furthermore, these excretions are not sent for diagnostic purposes but only to determine that the convalescent is not a bacillus carrier. Therefore, the cultural examinations in a large proportion of our cases are negative. It follows that for diagnostic purposes we must depend mainly on the serum reaction. Recently antityphoid vaccination has been coming into quite general use among the American troops. As is well known, a typhoid vaccination will cause a positive Widal reaction, and of late it has been becoming obvious to us that the more general use of antityphoid vaccination would largely vitiate any deductions in the future which could be drawn from positive Widal reactions in fevers of doubtful nature. For this reason we have decided to compile the results of our work to date, the possibility of typhoid vaccination having been excluded in all of the cases referred to below.

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[1] Major, Medical Corps, United States Army, president of the United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands.

While several articles from the pens of different authors have appeared on certain typhoid epidemics in the Philippines, (11) (12) (15) nothing, so far as we are aware, has been written which gives a comprehensive view of the typhoid situation throughout the Archipelago. Therefore, we feel that such a report can not fail to be of value to those interested in the sanitary problems of the Philippines.

TYPHOID IN THE TROPICS.

Typhoid fever, although not classed as a tropical disease, nevertheless is one which is of great interest and importance to those practicing in warm countries where it should always be born in mind when the physician is confronted by a patient with any febrile disturbance.

Manson says: "The existence of typhoid fever in the tropics was for long not only ignored but actually denied, even by physicians and pathologists of repute."(1) Malaria has been the scapegoat for the diagnostic shortcomings of the tropical practitioner. At the present day it is quite generally conceded that typhoid is a common disease among white men resident in low latitudes and that it is alarmingly prevalent among young Europeans in many parts of the tropical Orient. Castellani(2) and Rogers(3) agree with Manson in this opinion. Rogers found that among Europeans born and bred in the Tropics the incidence of the disease was especially marked in children under 15 years of age.

As regards the occurrence of enteric fever among natives of warm countries less is known. Castellani (2) states that it is common. He says: "In most cases the temperature does not run the typical course described in text books on general medicine, having sometimes a high remittent type, and at others an intermittent type, while cases of mixed infection with malaria are not rare." Rogers (3) found typhoid to be common among natives in India and believes it would be more frequently observed were it not that adult natives have acquired an immunity as a result of an attack in childhood. The sick native children are not often seen by competent attendants.

REPUTED OCCURRENCE OF TYPHOID FEVER IN THE PHILIPPINES.

In the early days of the American occupation of the Philippines it was the commonly accepted view that the occurrence of typhoid in these Islands was unusual.(10) That this opinion still has adherents is shown by the statements published in 1910 by Dr. Victor G. Heiser, Director of Health of the Philippine Islands. He says: "While cases of typhoid fever are undoubtedly contracted in the Philippines the disease can hardly be said to be prevalent here. To prevent its acquiring a foothold regulations were prepared * * *."(9)

For a number of years, especially since more settled conditions have allowed general resort to laboratory methods, the belief has been growing among medical officers of the army that typhoid fever is by no means infrequent in the Archipelago. It is our opinion that the disease is wide spread and very common in the Philippine Islands among both white men and natives.

Nichols in 1908 called attention to the frequent occurrence of typhoid fever among the Filipinos in the Visayas especially among the children.(11) Bruns, about the same time, stated that in his opinion typhoid was endemic in Iloilo.(12) Nichols also found typhoid fever among the natives at Taytay, a typical Tagalog town located about 32 kilometers from Manila.(13) La Garde(15) and Jackson(10) have both expressed the opinion that the frequency of typhoid among the natives is not sufficiently appreciated. Brownlee, formerly stationed at Malabang in Mindanao, states that he has found typhoid quite prevalent in Mindanao.(4) Dulin, after a long tour of duty in the Cotabato Valley, reached the conclusion that typhoid is endemic at all times and in all places in that portion of Mindanao.(4) Shockley, who has seen much service in Mindanao, confirms the above opinions as to the prevalence of the disease in that island.(4) Arlington Pond in a personal letter gives it as his opinion that there is much more typhoid in the Island of Cebu than is recognized. W. A. Powell from Lucena mentions the occurrence of typhoid among both adults and children. Huber states that he finds typhoid almost constantly present in the town of Bayambang, Pangasinan Province, Luzon.(14) Phalen, for two years president of this Board, voiced the belief that typhoid is an ever present endemic disease throughout the Philippines.(4) Many other medical officers of the army have verbally expressed the same opinion. Spanish and Filipino practitioners refer to the frequent occurrence of a "calentura", a fever of two or three weeks' duration which does not yield to quinine.

As will appear later, our laboratory findings confirm the above opinions as to the *wide range* of typhoid infection in the Philippines but can not, of course, throw much light on the *frequency* of its occurrence in the native population.

TYPHOID STATISTICS FOR THE PHILIPPINES FURNISHED BY THE BUREAU OF HEALTH.

Vital statistics in the Philippines, particularly outside Manila, are notably unreliable because of the scarcity of competent physicians and because of the great number of natives who are never seen by any medical attendant even in an illness terminating fatally. In view of the almost universal tendency to ignore typhoid fever and to consider malaria the cause of the doubtful fevers it is probable that the following figures, obtained from the Bureau of Health, do not err in the direction of exaggerating the death rate from enteric fever.

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Manila

TABLE I.—*Typhoid fever in the Philippines.*

Calendar year.	Number of deaths.	
	Manila.[a]	Provinces.[a]
1906	47	2,458
1907	77	2,133
1908	116	2,371
1909	90	2,387
1910	82	------ ------
Average	82.4	2,336

[a] The population of Manila in 1907 was 223,543, divided as follows: Americans and Europeans 9,079; Chinese 18,028; Filipinos 195,292; others 1,143. The population of the provinces referred to is approximately 6,434,390.

Granting a death rate of 20 per cent (which seems a liberal one and is much higher than the experience of the army among either white or native soldiers), the average of 82.4 deaths annually would indicate a yearly incidence of 412 cases of enteric fever in Manila or one *per* year for each 542 persons.

This average yearly rate of 82.4 deaths in a city of 223,543 people represents a mortality of 36.8 per 100,000. The Census bulletins for the registration areas in the United States show the death rate from typhoid per 100,000 to have been 25.3 for 1908, 30.3 for 1907, and 32.2 for the period 1901 to 1905.(22) The rate per 100,000 from 1901 to 1905 averaged 11.4 for Scotland, 11.2 for England and Wales, 13.1 for Ireland, 16.8 for Belgium, and 7.6 for Germany.

The following list shows the position of Manila as compared with certain large cities in Europe(23) and America.(22)

[The figures indicate deaths from typhoid per 100,000 of population.]

City.	Deaths.	City.	Deaths.	City.	Deaths.
1. Pittsburgh	129.6	8. MANILA	36.8	15. Omaha	20.3
2. Allegheny	110.1	9. Toledo	36.3	16. Milwaukee	18.1
3. Washington	56.6	10. Baltimore	35.8	17. New York	18.1
4. Philadelphia	52.3	11. Buffalo	28.9	18. London	12.3
5. New Orleans	40.9	12. Chicago	28.4	19. Hamburg	5.6
6. Memphis	37.8	13. San Francisco	27.0	20. Vienna	4.3
7. St. Louis	37.2	14. Boston	22.2	21. Berlin	3.8

As compared with American communities it will be seen that Manila corresponds with the middle group including such places as New Orleans, St. Louis, Toledo, and Baltimore. It is exceeded only by a few cities which are notorious for high typhoid rates. Its mortality record is far above that for the best American cities which themselves have an enormously greater typhoid rate than do many of the municipalities of Europe.

As regards the accuracy of the typhoid death rate in Manila, Doctor Andrews, in charge of the city morgue, states that he encounters many cases of typhoid at the autopsy of native bodies and that he considers the average of 82 deaths per year to be well within the limits. Most of the cases he finds have been undiagnosed or incorrectly diagnosed.

THE INCIDENCE OF TYPHOID AMONG AMERICAN SOLDIERS IN THE PHILIPPINES.

Considerable doubt may attach to the accuracy of the diagnoses in some of the deaths reported to the Bureau of Health as caused by typhoid, but in the case of the Army statistics for Americans during the past ten years this possibility of error is reduced to a minimum, since every soldier seriously ill is treated in hospital and the diagnosis in nearly all instances is made with the aid of laboratory facilities.

During the year 1898 typhoid was everywhere epidemic among the untrained volunteer troops in the United States. Throughout 1899 it decreased at the home stations but was widely prevalent in the Philippines among the great numbers of newly recruited volunteers who were undergoing the hardships of a strenuous campaign. These two years would show nothing of value when comparing the admission rates for troops serving in the Philippines and in the United States. Therefore, they have been omitted from the tables below which begin with the year 1900 and are compiled from the reports of the Surgeon General of the Army.

TABLE II.—*Typhoid fever in the United States Army, American troops.*

Calendar year.	Philippine Islands.					United States.				
	Mean strength.	Admissions.		Deaths.		Mean strength.	Admissions.		Deaths.	
		Number.	Per 1,000.	Num. ber.	Per 1,000.		Number.	Per 1,000.	Number.	Per 1,000.
1900	66,882	716	10.71	141	2.11	20,690	115	5.56	9	0.43
1901 (16)	59,526	315	5.29	58	0.97	26,115	250	9.43	17	0.64
1902 (17)	32,942	201	6.10	32	0.97	39,736	341	8.58	34	0.86
1903 (18)	18,671	82	4.39	15	0.80	42,264	246	5.82	12	0.28
1904	11,996	23	1.92	5	0.42	43,940	247	5.62	12	0.27
1905	11,057	31	2.80	3	0.27	42,834	153	3.57	13	0.30
1906	12,380	48	3.88	0	0.00	40,621	230	5.66	11	0.28
1907	11,699	41	3.50	3	0.26	35,132	124	3.53	7	0.19
1908	11,971	33	2.76	5	0.41	46,316	136	2.94	11	0.23
1909	12,844	79	6.15	2	0.15	57,124	173	3.03	16	0.28
Total	249,968	1,569	-------	264	-------	394,772	2,015	-------	141	-------
Average for 10 years	24,997	156.9	[a]6.28	26.4	[a]1.06	39,477	201.5	[a]5.10	14.1	[a]0.36

[a] Average admissions and deaths per 1,000 are based on the average of admissions and deaths for ten years as compared with average mean strength for same period.

For comparison with the above there is given below a table covering the same period and showing the number of admissions for malarial fevers and fevers of undetermined causation.

TABLE III.—*Malaria and undetermined fevers in the United States Army, American troops.*

Calendar year.	Philippine Islands.				United States.			
	Mean strength	Fevers undetermined, admissions per 1,000.	Malarial fevers.		Mean strength	Fevers undetermined, admissions per 1,000.	Malarial fevers.	
			Admissions per 1,000.	Deaths per 1,000.			Admissions per 1,000.	Deaths per 1,000.
1900	66,882	29.01	874.23	1.59	20,690	9.67	158.47	0.19
1901	59,526	25.38	501.62	0.65	26,515	8.22	109.07	0.00
1902	32,942	20.23	402.94	0.64	39,736	7.65	93.09	0.06
1903	18,671	(a)	451.83	1.26	42,264	(a)	57.46	0.00
1904	11,996	33.34	218.82	0.25	43,940	6.25	47.43	0.00
1905	11,057	35.27	258.84	0.54	42,834	6.91	43.84	0.02
1906	12,380	8.97	304.20	0.55	40,621	7.78	50.27	0.02
1907	11,699	15.73	167.79	0.17	35,132	4.67	30.20	0.00
1908	11,971	25.81	123.97	0.16	46,816	3.80	26.69	0.00
1909	12,844	25.46	112.35	0.15	57,124	2.15	23.06	0.00

[a] No data.

There was no mortality for the undetermined fevers shown in the Table III except in 1900 when it was 0.01 per 1,000 in the Philippines and 0.05 per 1,000 in the United States.

On studying these tables it will be observed that for the ten-year period the typhoid admission rate per 1,000 has been higher in the Philippines than in the United States, 6.28 as compared with 5.10. The death rate from typhoid has been three times as high in the Philippines, 1.06 per 1,000 as compared with 0.36. During the years 1907 and 1908, when the sanitary conditions and the service conditions in the Philippines closely approximated those at home, the typhoid admission rate has not materially differed in the two countries while in 1909 it was twice as high in the Philippines as in the United States. The admissions for 1910 have been 38, making an admission rate of approximately 3.1 per 1,000 which exceeds the home rate for 1908 and 1909.

In considering the comparative admission rates for typhoid in the Islands and at home, it is important to bear in mind that in the United States the water supplies of many towns and posts are not above suspicion, yet little care is generally exercised by soldiers to avoid drinking these doubtful waters. In the Philippines distilled water of unquestioned purity is everywhere furnished for soldiers and the fear of dysentery and cholera, together with the stringent orders on the subject, make the drinking of this water very general. If the same carelessness in drinking which we see everywhere at home were practiced here, we believe that the typhoid rate would be very much higher than it is at present.

A consideration of the above remarks points very strongly to a wide spread and easily accessible source of typhoid infection in these Islands.

That this source is also accessible to the native troops is shown by the following table:

TABLE IV.—*Admissions among Philippine (Native) Scouts of the United States Army for typhoid, malaria, and undetermined fevers.*

Calendar year.	Mean strength.	Admissions.				Deaths.[a]		
		Typhoid fever.		Malarial fever per 1,000.	Fever, undetermined, per 1,000.	Typhoid fever.		Malarial fever per 1,000.
		Number.	Per 1,000.			Number.	Per 1,000.	
1902	4,826	9	1.86	704.11	85.02	1	0.21	1.66
1903	4,888	5	1.02	522.09	0.00	1	0.20	2.45
1904	4,610	4	0.87	367.69	31.49	3	0.65	0.43
1905	4,732	7	1.48	571.01	15.64	2	0.40	0.81
1906	4,759	4	0.84	393.15	21.43	1	0.20	1.19
1907	4,679	6	1.28	312.67	19.45	0	0.00	0.43
1908	5,085	9	1.77	236.97	20.45	0	0.00	0.19
1909	5,369	7	1.30	203.95	17.69	1	0.18	0.00
Total	38,948	51				9		
Average for 8 years	4,619	6.38	1.36			1.13	0.24	

[a] There were no deaths from undetermined fevers.

Table IV shows that there has been a constant, though low, admission rate for typhoid among the Philippine Scouts since their organization. It is certain that the diagnoses for sick Scouts are less thoroughly worked up than are those of the white troops because of racial peculiarities and the difficulty of understanding their language, and also because of the fact that many of the Scout organizations are at small and isolated posts with meager laboratory facilities. Therefore, some cases of typhoid may lie hidden in the great mass of admissions for malaria and undetermined fevers, only the most characteristic cases being correctly diagnosed.

Since most of the Scouts are at posts where they come in contact with very few white men, it is almost certain that they pick up their infection at the post or in the country surrounding and that the disease is not imported from the United States as might be urged in the case of the white soldiers.

The lesser incidence of typhoid among the Scouts as compared with American troops corresponds with Rogers' experience with native troops in India. He attributes this difference to the influence of typhoid in childhood.

DISTRIBUTION OF TYPHOID FEVER IN THE PHILIPPINES.

The distribution of typhoid throughout the Archipelago, in so far as it has come under the notice of the Army medical officers, is shown in Table V.

TABLE V.—*Distribution of typhoid fever in the Philippines, Army statistics.*

Town or post.	Island.	Number of admissions for typhoid fever.								
		1904	1905	1906	1907	1908	1909	1910	1911 part.	Total.
Keithley	Mindanao		2	11	+	1				15
Marahui	do	5								5
Cotabato	do		(1)			(1)		2 (1)		2 (3)
Vicars	do	3								3
Overton	do						1	1	1	3
Malabang	do	2		1	+		4		1	9
Ludlow	do						51 (2)	22 (4)	(2)	73 (8)
Parang	do	1					(5)			1 (5)
Polloc	do								(2)	(2)
Field	do			3			1			4
Zamboanga	do	1	1	2	+					5
Jolo	Jolo					1				1
Balamban	Cebu					(1)				(1)
Cebu	do								(1)	(1)
Naga	do						1			1
Field	do		1							1
Downes	Leyte					3	1	1		5
Dagami	do				(2)					(2)
Field	do			1	13					14
Bumpus	do					11				11
Connell	Samar		3	2	+					6
Balangiga	do				(1)					(1)
Field	do		1							1
	Negros				1					1
Jossman	Guimaras	1	1	1	+		1	1		6
Iloilo	Panay				3					3
San Pedro	do			15	8	2				25

Mills	Corregidor						2 (1)	2	1 (1)	5 (2)
Stotsenburg	Luzon			1	3	3	1	2	1	11
Lucena	do							(3)		(3)
Gregg	do			7	+			(13)		8 (13)
Infanta	do				(1)					(1)
Wallace	do		3	1	+			(1)		5 (1)
San Fernando, Union	do							(19)		(19)
Pasay	do	1								1
Wilhelm	do							2 (1)	(1)	2 (2)
Manila Posts *	do	3	8		7	12 (1)	2 (1)	12 (3)	2 (2)	46 (7)
Imus	do						1			1
Daraga	do	2		2	+					5
Floridablanca	do								2	2
Bakoor	do	1								1
Los Baños	do						1			1
Bulan	do	1								1
Eldridge	do						10			10
McGrath	do	1					1	1	1 (1)	4 (1)
Bayambang	do							(2)		(2)
Cañacao	do								(1)	(1)
Pilar	do	1								1
Mariveles	Luzon						1			1
McKinley	do		8	1	2	4	2	2	1	20
Field	do		2							2
San Pablo	do								(1)	(1)
Malahi	do		1							1
Total		23	31 (1)	48	37 (4)	37 (3)	81 (9)	48 (47)	10 (12)	323 (76)

* Includes Division Hospital.

Numbers inclosed in parentheses () represent native cases. No statistics available for natives 1904–1906.

Spaces marked with a plus sign (+) indicate that one or more cases occurred at the locality indicated but the records do not show the number at many of the posts and give only the total for the year. In the horizontal totals the item marked with a plus sign (+) has been counted as one case only, consequently horizontal and vertical grand totals do not agree.

The figures in Table V were obtained from the reports of the Surgeon General of the Army, the records of the chief surgeon, Philippines Division, the records of this Board, and in a few instances from personal knowledge of members of the Board. The table does not pretend to be complete but it is sufficient to indicate how widely spread throughout the Archipelago is the infection with typhoid. The accompanying "spot map" graphically indicates the distribution. In considering Table V it must be borne in mind that the localities mentioned represent a majority of the places in the Islands where there are competent observers or laboratory facilities.

The Bureau of Health mortality statistics for the provinces cover all parts of the Archipelago except Mindanao, Jolo, and adjacent small islands. These reports show typhoid to be an extremely common cause of death in all of the important islands, the mortality rate in some provinces for certain years ranging from 50 to more than 160 per 100,000 of population. We have not gone into these figures in more detail because of the uncertainty which attaches to the diagnosis of typhoid, or any other disease for that matter, in the provincial reports.

WIDAL EXAMINATIONS ON THE BLOOD OF HEALTHY ADULT NATIVES.

With a view to determining how large a proportion of healthy natives might show the influence of antecedent typhoid, as evidenced by a positive Widal reaction, the Board has obtained the blood of 307 adult male Filipinos and the results are shown in Table VI. These examinations were made in 1910 and 1911.

TABLE VI.—*Widal examinations on the blood of healthy Filipino adults.*

Station or town.	Island.	Race or tribe.	Number.	Widal, dilutions about 1 to 20.		Widal, dilutions about 1 to 50.	
				Positive.	Negative.	Positive.	Negative.
Parang	Mindanao	Moro	12	1	11	1	0
Parang	do	Filipino	52	4	48	4	0
Camp Wilhelm	Leyte	Visayan	12	0	12		
Camp James	do	do	12	0	12		
Cotabato	Mindanao	Ilocano	12	0	12		
Camp Wallace	Luzon	Visayan	12	0	12		
Regan Barracks	do	Ilocano	12	2	10	0	2
Camp Connell	Samar	Visayan	12	2	10	0	2
Isabela	Basilan	Moro	12	0	12		
Bojelebung	do	do	6	0	6		
Bumpus	Leyte	Visayan	12	1	11	0	1
Fort Mills	Corregidor	Macabebe	36	4	32	2	2
Camp Gregg	Luzon	Visayan	40	0	40		
Camp Hay	do	Igorot	20	2	18	0	2
Camp Hayt	Samar	Cagayan	12	4	8	2	2
Kaligton	Luzon	Ilocano	13	0	13		
Fort Mills	Corregidor	Visayan	20	0	20		
Total			307	20	287	9	11
Percentage				6.5	93.5	2.9	

Reactions in 1 to 50 dilution were done only on those cases which proved postive in 1 to 20 dilution.

There are few data available to indicate what proportion of healthy men in any country might give a positive serum reaction from causes other than antecedent typhoid. The length of time that the Widal reaction persists after convalescence from enteric fever is variable, ranging from six weeks to twenty years, the long continuance in some cases perhaps being due to bacilli persisting in the gall bladder. The fact that 6.5 per cent of the adults we tested showed a positive reaction in 1 to 20 dilution and 2.9 per cent in 1 to 50 dilution probably points to an antecedent attack of typhoid in some of these individuals and, when taken with the statistical evidence already presented, is suggestive of a wide spread distribution of the disease in the native population.

THE INFREQUENCY OF SEVERE AND FATAL EPIDEMICS AMONG NATIVES.

The above quoted statistics and examinations indicate to our minds that typhoid is very generally present in the Philippines. The water supplies are almost universally bad, the proper disposal of excreta is almost entirely neglected, the crowding in the habitations and the native manner of eating favor contact infection. Yet, in spite of these unfavorable conditions, there is little evidence that severe and destructive epidemics of typhoid fever occur among the Filipinos. The relative scarcity of typhoid among the natives in India is thought by Rogers to be due to immunity acquired in childhood. Nichols(11) expresses the opinion that typhoid is prevalent among children in the Philippines. To determine if evidences of previous typhoid in Filipino children could be detected, we performed Widal reactions on the blood of 284 healthy school children. The results are shown in Table VII and are entirely negative except for three cases at Ormoc, a town where we had good reason to believe that typhoid had recently prevailed.

TABLE VII.—*Widal examinations on the blood of healthy Filipino school children.*

Town.	Island.	Race or tribe.	Number.	Widal, dilutions about 1 to 20.		Widal, dilutions about 1 to 50.	
				Positive.	Negative.	Positive.	Negative.
Baguio	Luzon	Igorot	45	0	45		
Paranaque	do	Tagalog	30	0	30		
Lucena	do	do	50	0	50		
Ormoc	Leyte	Visayan	14	3	11	1	2
Malabang	Mindanao	Filipino	7	0	7		
Parang	do	do	30	0	30		
Iligan	do	do	22	0	22		
Jolo	Jolo	Moro	36	0	36		
Floridablanca	Luzon	Filipino	26	0	26		
San Nicolas	do	do	24	0	24		
Total			284	3	281	1	2
Percentage				1.0	99.0	0.3	

It will be seen that the percentage of positive results was much less than among adults. These children ranged from four to thirteen years of age and the majority were under eight years old. If typhoid were common among the Filipino school children, it is probable that the percentage of positive reactions among them would be considerable. It is possible, of course, that the disease may occur widely among infants and that the agglutinating influence in nearly all cases had passed away before the time at which we saw these children. However, such an explanation does not appear to us probable.

Our clinical records, to be discussed later, indicate that a considerable number of the cases which give positive laboratory finding present clinical appearances entirely different from the classic description of typhoid fever. Patients with a short course, little fever and few symptoms, were found commonly among both Americans and Filipinos. It is by no means unlikely that such cases throughout the native population are very common and that the true nature of the malady is overlooked. The disease in this form can be diagnosed by laboratory procedures alone, and it is only by working along this line that the true incidence of typhoid fever among the Filipinos can be determined.

INFLUENCE OF SEASON ON PREVALENCE OF TYPHOID.

In Europe and America typhoid fever may occur at any season, but its incidence is greatly increased in August, September, and October. In India, as a whole, Roberts has shown that the disease is very generally distributed throughout the year as follows: first quarter 18.1 per cent; second quarter 31.3 per cent; third quarter 28.1 per cent; fourth quarter 22.4 per cent. The experience for four years (the only ones for which monthly records are available to us) shows that in the Philippines the disease is distributed throughout the year in a quite regular manner.

TABLE VIII.—*Incidence of typhoid fever by months in the Philippines.*

Month.	1904	1908	1909	1910	Total.	Quarter.	
						Number.	Per cent.
January	2	3	1	7	13	78	24.61
February	1	5	7	10	23		
March	1	13	7	21	42		
April	1	8	1	2	12	44	13.88
May	0	9	2	0	11		
June	5	14	2	0	21		
July	4	25	5	4	38	90	28.39
August	2	23	8	0	33		
September	1	14	3	1	19		
October	3	12	43	2	60	105	33.12
November	1	17	3	0	21		
December	2	13	5	4	24		
Total	23	156	87	51	317		

The considerable increase in the last quarter may be considered accidental since the 43 cases in October, 1909, all occurred at one post, the Ludlow Barracks epidemic.

The season varies considerably in the Philippines in different localities and for different years, but in general it may be said that the period from December 1 to June 1 represents the dry season.

Since our military patients mainly are drawn from a population consisting almost exclusively of young adult males, it has been impossible for the Board to draw any conclusions as to the influence of sex or age on the incidence of typhoid in the Philippines except in so far as we have mentioned the latter factor in the paragraph below Table VII.

PART II. LABORATORY INVESTIGATIONS OF TYPHOID FEVER IN THE PHILIPPINES.

During the two and one-half years that the Board has been working on the subject of typhoid fever the following examinations have been made in the case of persons having typhoid fever or suspected of having it.

TABLE IX.—*Examinations by the Board on persons suspected of having typhoid, December 1, 1908 to April 30, 1910.*

Results.	Widal tests.	Cultures from—			
		Stools.	Urines.	Blood.	Bile.
Positive	192	19	9	17	1
Negative	261	498	320	139	0
Total	453	517	329	156	1

The 453 serum reactions were performed on the blood of 369 persons. The 192 positive Widal reactions were made on the blood of 177 persons, of whom 118 were Americans and 59 were Filipinos. The 517 stool cultures were made on 199 individuals, the 329 urine cultures on 164 individuals, and the 156 blood cultures on 141 individuals.

BLOOD CULTURES, 17 POSITIVE, 139 NEGATIVE.

The organisms were sought in the blood by placing it in sterile bile, incubating for twenty-four hours and plating on Endo medium. Many of our specimens of blood were very old and the amount small, which accounts partially for the small number of positive findings. The fact that the disease in many instances was far advanced needs also to be considered. All of our positive blood cultures were obtained from patients in the Division Hospital or at posts we visited, and from these patients we obtained a large amount of blood and placed it at once in the bile medium. Since all of these cases came under our immediate observation, we feel sure that the days of the disease, as shown in the following table, are approximately correct.

TABLE X.—*Days on which positive blood culture was obtained.*

Day.	Number positive.	Day.	Number positive.
Second	1	Twelfth	1
Third	1	Thirteenth	1
Sixth	2	Fourteenth	1
Seventh	2	Unknown	1
Eighth	3	Total	17
Ninth	3		
Eleventh	1		

Among the cases which showed bacilli in the blood at the end of the first week or later the following are of interest:

TABLE XI.—*Relation of Widal reaction to positive blood culture.*

Case.	Day.		
	Blood positive.	Widal negative.	Widal positive.
3	Fourteenth	Twelfth	Fourteenth.
4	Seventh	Seventh	Ninth.
258	Eighth	Sixth	Ninth.
W.	Seventh	Sixth and tenth	Twenty-first.
B.	Eighth	Seventh	Eighth.
T.	Twelfth	Twelfth	(?)
402	Sixth	Sixth and seventh	Twenty-second.

STOOL CULTURES, 19 POSITIVE, 498 NEGATIVE, AND URINE CULTURES, 9 POSITIVE, 329 NEGATIVE.

Stool and urine specimens were smeared over the surface of Petri dishes containing Endo medium. Cultures from the excreta were rarely employed for diagnosis. Nearly all of those shown in the table were made during convalescence to detect possible carriers, three negative cultures at six-day intervals being required before a soldier was returned to duty. These facts account in the main for the low proportion of positive findings. Another factor which must be considered is that the stools and urines in many cases had come a long distance. Our experience, both practical and experimental, has been that after a few days no typhoid bacilli can be obtained by cultural methods from a stool, whether naturally or artificially inoculated with *Bacillus typhosus.*

CHARACTER OF TYPHOID ORGANISM ISOLATED IN THE PHILIPPINES.

At the end of 1908 this Board had found the blue strain of typhoid in 5 patients in the Philippines and expressed the opinion that the prevailing type of organism in the Philippine Islands might be one which produced primary acidity in litmus milk followed after several days by alkalinity. Further experience has not confirmed this view. During nearly two years we have isolated only one blue typhoid organism from either a native or a white man, and recently, on re-testing a number of organisms, isolated in the Philippines, all except that one failed to give the blue reaction. Therefore, it is, probable that the blue strain occurs with no greater degree of frequency in the Philippine Islands than in the United States. The distinction in type is probably of no importance and the variations reported may at times be due to the use of milks containing variable quantities of monosaccharid. In fact, acidity followed by alkalinity is given as the type for typhoid by Hiss and Zinsser.(20)

It appears that there is no constant difference, either in their litmus-milk reaction or in their behavior in the presence of immune sera, between the strains of *Bacillus typhosus* found here and those met with in the United States.

WIDAL EXAMINATIONS, 192 POSITIVE, 261 NEGATIVE.

The 192 positive examinations were made on the blood of 177 individuals of whom 5 had received antityphoid vaccination and two others gave a clear history of previous typhoid, leaving 170 cases in which the positive reaction was of diagnostic value.

Only the microscopic method was employed by us in making the agglutination test, both loss of motility and clumping being required before making a positive report. A dilution of 1 to 50 was used and one hour allowed to elapse before recording the result. Part of these tests were made with fluid blood and the remainder with dried blood, the dilution in the latter cases being, of course, only approximate.

Nichols(11) and Bruns(12) in the Islands of Leyte and Panay investigated two epidemics in which they found an absence of the Widal reaction until very late in the disease. In 11 cases Nichols found it absent on an average till the twenty-fifth day. In the 177 positive Widal reactions obtained by us we were often dependent on others for the statement of the day of the disease. As thus given, the figures are as follows:

TABLE XII.—*Days on which positive Widal reactions were obtained.*

Day.	Number.	Day.	Number.	Day.	Number.	Day.	Number.
Second	13	Eleventh	7	Twentieth	1	Forty-fifth	1
Third	13	Twelfth	1	Twenty-first	3	Fifty-eighth	1
Fourth	12	Thirteenth	5	Twenty-third	1	Seventy-third	1
Fifth	14	Fourteenth	3	Twenty-fifth	1	Unknown [a]	35
Sixth	8	Fifteenth	2	Twenty-sixth	3	Total	177
Seventh	14	Sixteenth	2	Twenty-seventh	1		
Eighth	12	Seventeenth	1	Thirtieth	2		
Ninth	6	Eighteenth	3	Thirty-sixth	1		
Tenth	5	Nineteenth	3	Thirty-seventh	2		

[a] This "unknown" group contains 5 positive Widals obtained in persons who had been vaccinated against typhoid and who did not have the disease, 2 obtained in persons not suffering from typhoid but giving a clear history of a previous attack, a few from whom we never received records, and a series of 20 natives found at San Fernando who undoubtedly had the disease but from whom no history was obtainable.

From a study of the temperature charts of the patients we feel sure that many of the cases were somewhat further along in the disease at the time of the appearance of the Widal reaction than would be indicated by the above figures. On the other hand, most of the cases reported above as having a very late serum reaction undoubtedly would have shown one earlier, but none was done till the date indicated in the table. In a series of cases in which a negative followed by a positive reaction was obtained and in which the onset of the disease has been fixed with reasonable accuracy from the clinical records we found no notable prolongation of the pre-agglutination stage. The figures appear in Table XIII.

TABLE XIII.—*Days on which Widal appeared in patients who had previously given a negative reaction.*

Case number.	Day.		Case number.	Day.	
	Widal negative.	Widal positive.		Widal negative.	Widal positive.
5	Sixth and seventh	Eighth.	117	Seventh and eighth	Tenth.
21	Tenth	Eleventh.	108	First and third	Fourth.
7	Seventh	Eighth.	138	Sixth	Seventh.
4	Seventh	Ninth.	131	Second	Fourth.
3	Twelfth	Thirteenth.	128	Second	Fifth.
258	Sixth	Ninth.		Seventh	Eighth.
1076	Sixth	Seventh.	213	Fifth	Tenth.
350	Second	Fourth.	402	Sixth and seventh	Twelfth.
352	Seventh	Eighth.			
383	Fifteenth	Twenty-fourth.		Average	Ninth.
137	Fourth and seventh	Tenth.			

From our 89 characteristic cases of typhoid fever, analyzed below and in which the date of onset could be quite definitely fixed, we obtained positive Widal reactions on or before the tenth day of the disease in 72 per cent and on or before the fifteenth day in 87 per cent. In all but one of the remaining 13 per cent no serum test had been performed prior to the fifteenth day, and it is probable that all or nearly all were positive before that time.

From the above figures it is evident that the experience of the Board *does not indicate any general tendency toward unusual delay in the development of agglutinins in the blood of patients suffering from typhoid fever in the Philippines.*

We have analyzed the clinical records of over 100 patients with elevations of temperature and for whom negative Widal reactions were obtained. In only one instance was there the slightest reason to believe that the febrile condition was enteric fever, and in this single case the temperature curve and symptoms were not entirely characteristic. Repeated typhoid serum reactions were negative up to the fourteenth day, after which time no specimens were submitted. Serum reaction with *Bacillus paratyphosus* was reported negative but fell in the class of reactions which some observers report as "partial." The leucocyte count was 9,000 and there was 33.8 per cent of small lymphocytes and 9.2 per cent large mononuclears. No pigmented leucocytes were seen. A relapse occurred in this patient which promptly yielded to hypodermic use of quinine although previous examinations for parasites and previous use of quinine by mouth had resulted negatively. The true nature of this case remains in doubt.

TYPHOID BACILLUS CARRIERS.

Failure attended our efforts to detect carriers as a result of 517 stools cultures made on 199 individuals and 329 urine cultures made on 164 individuals. However, we discovered one typhoid bacillus carrier by accident while examining for dysentery the stool of a patient who had been having loose movements of variable frequency for nearly 4 months.

The patient, aged 30, gave no history of typhoid fever. He had been in the Philippines one year and had ten years' service in the Army. He had dysentery in 1907. The last part of June, 1910, while on a mapping detail in the field, he developed a very severe attack of diarrhœa with some blood and mucus. Did not go on sick report and diarrhœa persisted. Lost about 28 pounds in weight. On admission to Division Hospital September 5, 1910, he was having about 4 stools daily, usually liquid and yellowish or clay-colored. They contained neither blood nor mucus. During his stay in the hospital from September 5 to October 14, 1910, his temperature never went above 37°.4C. and was generally normal. Pulse averaged 90 at times reaching 110. Repeated examinations of the stools for ova and amœbæ were negative.

The first stool culture was made on October 13, the day before he sailed for the United States and about 110 days after his original attack of diarrhœa. It was positive for *Bacillus typhosus*. Meantime the patient had sailed for the United States, but by telegraphic request we obtained a specimen of his blood while the transport was at Mariveles and this gave a positive Widal reaction.

Under date of November 19, 1910, the commanding officer of the General Hospital at Presidio of San Francisco notified us that the soldier was still excreting typhoid bacilli.

As far as we can learn this is the only carrier which has been found in the Philippines.

PARATYPHOID FEVER AND GROUP AGGLUTINATION.

Strong in 1902 reported the recovery at autopsy of a paracolon organism from a soldier who had died with the usual symptoms of typhoid.(21) Ruediger, at the Bureau of Science, recently isolated a para-typhoid strain from the blood of a dog which died of rabies and showed also a purulent pneumonia. Our experience with the paratyphoid organism is confined to one case. The patient, who also had tuberculosis, ran a febrile course of eighteen days' duration, the curve suggesting mild typhoid fever. There were the usual premonitory symptoms of typhoid fever, and the progress of the disease was characterized by constipation, tympanites, pain in head and neck, splenic tumor, and normal leucocyte count. From the blood was obtained a pure culture of *Bacillus paratyphosus* "A." The blood serum at first agglutinated both *Bacillus typhosus* and *Bacillus paratyphosus*. At a later date it failed to react with the former organism.[2]

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[2] Since this writing we have isolated *Bacillus paratyphosus* "A" from the blood of a Japanese with a continued fever at Camp Stotsenburg.

We have tried the agglutination reaction with *Bacillus paratyphosus* on a considerable number of patients, and aside from the above have obtained positive reactions only twice, one of these sera also reacting with *Bacillus typhosus*. A more detailed report of the above case and a discussion of the subject of group agglutinations is contained in our quarterly report for December 31, 1910.

OBSERVATIONS ON THE BLOOD OF TYPHOID FEVER PATIENTS.

The blood of all of the patients considered was examined for malarial organisms with negative results except as noted below under complications. No other blood work was done on the cases except leucocyte counts, most of which were made at the laboratory of the Division Hospital on patients who were being treated in that institution. Thirty-six white blood counts were made on the blood of 19 patients and 31 differential counts on 15 of the same individuals. The average results divided as to race, appear below:

TABLE XIV.—*Average white blood counts on typhoid fever patients.*

Average.	Leucocytes per c. mm.	Polymorphonuclears.	Small lymphocytes.	Large lymphocytes.	Tranitionals.	Eosinophiles.	Mast cells.
Americans	6,540	66.2	25.7	6.3	1	0.6	0.1
Filipinos	10,234	66.9	26.3	5.3	0.3	0.5	0.7
Both races	7,669	66.5	25.9	6	0.8	0.6	0.3

These counts were made in the first week or ten days of the disease and do not show a tendency to the usual leucopenia, the average being normal for the white men and rather above normal for the natives. The lowest count for a native was 6,300 and the highest 15,400. For the Americans ten counts were below 6,000, fourteen between 6,000 and 10,000, and one was 11,500.

Rogers in a study of typhoid in India found the small lymphocytes to be increased above the normal maximum of 30 per cent in over one-half his patients. In our series this was not the case, in fact we should consider the average of 25.9 per cent as low, since it has been our experience that in the Philippines the count of the small lymphycytes is uniformly high in healthy men, usually ranging above 30 per cent. This is true for natives, and for Americans with one or more years of tropical service.

The low eosinophile count in this native series is very unusual and probably is due to the fact that most of the patients were Scouts who had been relieved of their intestinal parasites prior to acquiring typhoid fever.

PART III. CLINICAL ASPECTS OF TYPHOID FEVER IN THE PHILIPPINES.

After excluding vaccinated patients, persons with clear histories of previous typhoid and one case of paratyphoid there remain the clinical histories of 157 patients sick during the last two and one half years and for whom the laboratory findings indicated the existence of typhoid infection. For clinical analysis we have divided these cases into four groups as follows:

Group I; 7 cases terminating fatally; 4.5 per cent.

Group II; 19 cases with short ill-defined fever of less than 10 days' duration; 12.1 per cent.

Group III; 41 cases of irregular fever of less than 10 days' duration, probably representing last part of mild ambulant typhoid types; 26.6 per cent.

Group IV; 89 cases of well marked continued fever, clinically typhoid, lasting more than 10 days; 56.6 per cent.

GROUP I. CASES TERMINATING FATALLY.

Seven patients out of the 157 died, a mortality of 4.5 per cent. The cause of death was peritonitis following perforation of the intestine in 3 instances, toxæmia in 3, and shock following hæmorrhage in one. *Bacillus typhosus* was obtained from the blood of 3 of these fatal cases and from the gall bladder of another.

Rogers found a mortality of 16.3 per cent among 129 cases in Calcutta and reports that in the British army in India the returns show a death rate of 26 to 27 per cent at the present time (1909–1910). This indicates a more serious condition than is seen in these Islands. The range in the typhoid mortality experience of the Army in the Philippines for the last ten years as contrasted with that among the troops in the United States is shown in Table XV:

TABLE XV.—*Mortality for typhoid fever in the Army in the Philippines and United States.*

Calendar year.	In the Philippine Islands.						In the United States.		
	American troops.			Filipino troops.			American troops.		
	Cases, number.	Deaths, number.	Mortality, per cent.	Cases, number.	Deaths, number.	Mortality, per cent.	Cases, number.	Deaths, number.	Mortality, per cent.
1900	716	141	19.69				115	9	7.83
1901	315	58	18.41				250	17	6.80
1902	201	32	15.92	9	1	11.11	341	34	9.97
1903	82	15	18.29	5	1	20.00	246	12	4.88
1904	23	5	21.74	4	3	75.00	247	12	4.86
1905	31	3	9.68	7	2	28.57	153	13	8.50
1906	48	0	0.00	4	1	25.00	230	11	4.78
1907	41	3	7.32	6	0	0.00	124	7	5.65
1908	33	5	15.15	9	0	0.00	136	11	8.09
1909	79	2	2.53	7	1	14.29	173	16	9.25
Total	1,569	264		51	9		2,015	142	
Average			[a]16.83			[a]17.65			[a]7.05

[a] The average mortality was obtained by dividing the total number of deaths for the period under consideration by the total number of cases for the same period.

It will be observed that the average mortality is a trifle higher for the Filipino than for the American troops serving in the Philippines, 17.6 per cent as compared with 16.8 per cent. It is quite probable that the death rate would be lower if every case of mild enteric fever occurring among the Scouts received the appropriate diagnosis. Rogers found a death rate of 26 per cent among 50 native Indian cases. Among the 26 Filipino cases for which we received clinical histories only one died, a mortality of 3.8 per cent. The average mortality for ten years among the American soldiers in the Philippines is very much higher than at home, 16.83 per cent compared with 7.05. This can be attributed mainly to the large amount of typhoid with a high mortality in the first four years of the decade, a time when the facilities for nursing, feeding and sheltering the patients were less favorable than in the United States. During the last six years, when the conditions in the Philippine hospitals have approximated those of similar institutions in the United States, the mortality has been no higher than at home (255 cases, 18 deaths, mortality 7.1 per cent). *Therefore, it seems safe to conclude that the type of typhoid infection now being encountered in the Philippines is, under present conditions, no more fatal to the American than that met with in the United States.*

GROUP II. CASES WITH ILL-DEFINED FEVER OF LESS THAN TEN DAYS DURATION.

Nineteen out of the 157 clinical records (12.1 per cent) show a slight febrile movement lasting less than ten days with few symptoms and nothing characteristic on which to base a diagnosis. The true nature of these cases could be determined only by laboratory methods. In addition to finding a clear-cut positive Widal reaction in all of the nineteen we were able to obtain by cultural methods the *Bacillus typhosus* from the stools of three and the urine of one other of this group.

GROUP III. CASES WITH IRREGULAR FEVER OF LESS THAN TEN DAYS, DURATION, PROBABLY REPRESENTING THE LAST PART OF LATENT OR AMBULANT TYPHOID TYPES.

Forty-two cases out of the 157 (26.6 per cent) presented an irregular temperature chart which resembled the last week or ten days of the classical typhoid curve. These patients were, in our opinion, suffering from a mild ambulant form of typhoid fever and came under observation only late in the disease. Such cases were particularly numerous in the Ludlow Barracks epidemic, constituting 33 per cent of the admissions, and many of them probably never would have come on sick report had it not been for the fact that the temperatures of all soldiers were being taken daily and every one with fever was placed in hospital for observation.

In addition to obtaining positive serum reactions in all of this group we isolated *Bacillus typhosus* from the stools of 5 patients and from the urine of another.

GROUP IV. CASES WITH WELL MARKED CONTINUED FEVER LASTING MORE THAN TEN DAYS AND CLINICALLY TYPHOID.

In this group there are 89 cases (56.6 per cent of the entire 157) which ran a febrile course of ten days or more and which in nearly every instance would be considered typhoid fever from clinical observations alone. The diagnosis was confirmed in each of these cases by the laboratory findings which for the great majority were positive Widal reactions and for the remainder cultures of *Bacillus typhosus* obtained from the blood or excreta. There were 4 positive urine cultures, 5 positive stool cultures and 15 positive blood cultures from this group.

Based on clinical appearances we divided these cases as follows; typical, severe, 18 (20.2 per cent); typical, moderate, 46 (51.7 per cent); typical, mild, 10 (11.2 per cent); atypical, mild, (lacking a fastigium) 12 (13.5 per cent); atypical, severe, 3 (3.4 per cent). The clinical histories for many of these cases are deficient as regards the presence or absence of the less important symptoms and signs, therefore in the analysis which follows the percentage of occurrence for some of the minor features will be an underestimate. With all the clinical records there were well kept temperature charts which rendered easy a correct estimate of the febrile type of the disease.

Onset of the disease.—The onset was sudden in 4 cases and gradual in the remaining 85. The premonitory symptoms included headache, backache, pains in bones and joints, lassitude, anorexia, diarrhœa, abdominal pain, nausea, vomiting, chills, fever, stiff neck, vertigo, and myalgia. They did not differ from those commonly encountered in the United States. Chills or chilliness were recorded in 10 cases.

Febrile course.—The onset of the disease could be fixed with reasonable accuracy in all of these 89 cases. The lengths of the febrile reaction, collected in groups each covering a 5-day period, are shown below.

TABLE XVI.—*Duration of febrile course for 89 characteristic typhoid cases.*

Days.	Number.	Per cent.	Days.	Number.	Per cent.
Between 10 and 15	8	8.98	Between 31 and 35	9	10.11
Between 16 and 20	24	26.97	Between 36 and 40	10	11.24
Between 21 and 25	21	23.60	Over 40	4	4.49
Between 26 and 30	13	14.61	Average, 25.1.		

The cessation of fever in all cases has been arbitrarily taken as the date after which the evening temperature did not rise above 37°.2 C. We believe that the above figures represent the minimum of febrile course and that possibly the real average was slightly longer as a result of the patients having been taken sick somewhat earlier than appeared upon the records. The average for our series was 25.1 days. For comparison may be mentioned the 780 cases studied by the Spanish-American

War Commission where the average duration was 10.5 days(19) and McCrae's large series in America where the average was 31 days.

The initial step-like increase of temperature characteristic of the early days in typhoid fever was rarely seen, the patients usually being well along the first week when they came under observation. A clearly marked fastigium was the rule, being present in 71 cases, 79.7 per cent of the series. For 18 persons a definite fastigium was lacking, the morning and evening excursions of temperature being pronounced even at the height of the disease. The maximum range of fever is shown below.

Never up to 39°.4 C., 9 cases. Never up to 40°.5 C., 45 cases.
Never up to 40° C., 26 cases. Never up to 41°.1 C., 9 cases.

The decline of the fever was characterized by gradually increasing remissions, such as are observed in typhoid in the United States, and no chart was found showing the unduly prolonged intermittent stage described by Rogers in India.

Recrudescences and relapses.—Recrudescences occurred in 4 cases (4.5 per cent) the lengths of the febrile disturbances being respectively six, seven, ten, and eleven days. Relapses occurred in 7 cases (7.9 per cent) lasting nine, twelve, fifteen, sixteen, seventeen, twenty-four, and twenty-five days, respectively. One patient was desperately ill during the relapse.

Pulse rate.—That the pulse rate was slow as compared with the temperature elevation was the almost universal experience, the disease in this respect conforming to the classical type in Europe and America. The low pulse rate was especially commented on by Lieutenant Dulin in the Ludlow Barracks epidemic where it rarely rose above 100 and was often normal at the height of the disease.

A dicrotic pulse was recorded in 13 out of the 89 cases, (14 per cent).

Symptoms of intestinal origin.—Constipation was a marked feature in this series. Diarrhœa has been credited to each patient who showed abnormal frequency for the evacuations at any time during the disease but there were only 21 such cases out of 89, an incidence of 23 per cent. Fifty-one patients (57 per cent), were constipated and, in 13 (14 per cent), the bowel movements were of normal frequency. In 4 cases there was no record. The percentage with diarrhœa is slightly higher than that found by McCrae in his American series (17 per cent) but considerably lower than that met with by Rogers in India, where 63 per cent showed diarrhœa at some period of the disease. Curschmann in Berlin found 73 per cent to have diarrhœa at some stage of the disease.

Since diarrhœa is considered of bad prognostic significance(3)(19) it may be that its low incidence among our patients is related to the low death rate of 4.5 per cent which was met with in this series.

Abdominal pain was noted as present in 13 of the 89 characteristic non-fatal cases, (14 per cent), was absent in 2 and there was no record for the remainder. Tympanites was recorded for 21 patients, (23 per cent), was absent for 7, and for the remaining 61 there was no mention of this symptom. Vomiting at some time during the disease occurred in 13 cases, (14 per cent).

Hæmorrhage was noted in 5 instances, 5.6 per cent of the entire group. Also it was the cause of death in one of the fatal cases not included in the series of 89. This percentage corresponds closely with that found by Curschmann in Berlin and Osler in Baltimore but is much lower than the experience of Rogers who records hæmorrhage in 17 per cent of his Indian series. Perforation was seen only in the fatal cases, occurring three times.

Symptoms referable to the nervous system.—Headache is recorded in 40 cases, or 45 per cent. Delirium was noted for 10 patients, (11 per cent), stupor or coma for 7 (8 per cent), and subsultus tendinum in 2 (2 per cent). Under this heading may be mentioned the complications occurring in four cases, two having developed post-febrile psychosis, one a multiple neuritis of the lower extremities and the fourth a condition of cardiac and nervous irritability.

Miscellaneous signs and symptoms.—Rose spots were noted as present in 26 cases (29 per cent), absent in 6 (7 per cent), and in the remaining histories there is no mention. The spleen was enlarged in 18 cases (20 per cent), not enlarged in 11 (12 per cent), and in the remaining cases the records are silent on this point. Cough was present in 10 cases and bronchitis is recorded in three.

Urine.—Albumen was present in the urine of 13 patients (15 per cent), was absent in 19 (21 per cent). Casts were present in 8 cases (9 per cent) and absent in 19 (21 per cent). In the remaining cases there were no records of the urinary examinations.

Complications and sequelæ.—Out of the 89 non-fatal cases the following complications and sequelæ were recorded; malarial fever, twice; catarrhal otitis media, once; phlebitis of the internal saphenous, once; bronchial spirochetosis, twice; multiple neuritis of the lower extremities, once; post-typhoid psychosis, twice; cardiac and nervous irritability, once; intestinal hæmorrhage, five times.

Among the fatal cases, Group I. it will be recalled there were 3 cases of perforation and one of hæmorrhage.

TYPHOID FEVER AMONG FILIPINOS.

We have performed laboratory tests on 105 Filipinos suspected of having typhoid fever and obtained positive serum reactions in sixty. *Bacillus typhosus* was recovered from the stools of three of these and from the blood of two. Twenty-one of the positive results were obtained from the San Fernando epidemic, and no case histories are available. From the remainder of the 60 we received clinical records for 26 patients of whom 1 died with perforation, 1 ran an entirely atypical course, 12 apparently were received in the last week of a classical typhoid attack while the remaining 12 passed through a period of continued fever ranging from ten to thirty-nine days in length and averaging 29.6.

These last 12 cases, which on clinical grounds alone appeared to be typhoid, are included in our Group IV. considered above, but will be briefly touched on at this point in order to determine if the disease in natives differs from that seen in the white man. All but two of these natives ran a febrile course of over 19 days. The onset was gradual in all instances. Chills were noted for two patients. The temperature chart showed a well defined fastigium in seven cases and the fever ranged at the height of the disease between 40° and 41.°1C. Nose bleed occurred in three, rose spots in two, marked abdominal pain in four and tympanites in 7. Diarrhœa was noted in 4, constipation in 5 and there was no record as to bowel movements in 3. As complications, hæmorrhage occurred in two cases, post-typhoid psychosis in one and bronchial spirochetosis in two

From a study of these cases it can be said that they did not differ from typical typhoid fever of moderate and severe type as seen among white men in the United States or in the Philippines.

The atypical cases which constituted so large a proportion of the Filipino admissions in the Camp Gregg epidemic and occasionally elsewhere are of special interest because of their probable rôle in spreading and keeping up infection. We have not had enough of them under observation to make any analysis of their symptomatology profitable. Rogers found such types in 17.8 per cent of his patients in India, this percentage being much lower than was seen in our series. How commonly these atypical cases occur in the general native population is one of the unsolved problems of tropical medicine.

Summing up the clinical aspects, we found that more than one-third of the admissions were atypical and that the well marked cases of typhoid in the Philippines, whether the patients were white or native, differed in no essential respect from the same disease as seen in the United States and Europe. The experience corresponds in general with the findings of Rogers in India. The atypical cases were about equally common in the two races.

RELATION OF BACILLUS TYPHOSUS TO THE SO-CALLED UNDETERMINED FEVERS.

It seems to us that the most important point brought out by the clinical study of the cases is the frequency of occurrence in the Philippines of the short irregular and atypical forms of typhoid fever. Groups II. and III. constituted 38.7 per cent of the entire 157 cases of typhoid infection. These two classes of mild infections formed 45.6 per cent of the total admissions at Ludlow Barracks and 69.2 per cent of the admissions among the Scouts at Camp Gregg. Without laboratory examinations or the presence of a wide-spread epidemic, such as prevailed at Ludlow Barracks, few, if any, of these mild enteric fevers would have received a correct diagnosis. This clinical type of infection with *Bacillus typhosus* may account for a considerable part of the so-called undetermined fevers met with in the Tropics, the prevalence of which in our army is shown in Tables III and IV. The sanitary importance of detecting such atypical typhoid cases is obvious.

Throughout our analysis we have been on the look-out for cases corresponding with the description of Brill's disease but have seen none suggesting that condition or the recently described Manchurian typhoid.(24)

DIAGNOSIS, PROGNOSIS, AND TREATMENT.

In the well-marked cases of typhoid in the Philippines the diagnosis can be made by the usual symptoms and physical signs recognized in temperate climates. In the large group of short atypical fevers diagnosis is impossible without resort to laboratory procedures. The prognosis with favorable facilities for treatment is good, the mortality for the last few years, as shown above, being about 7 per cent and no higher than was seen in the United States. The study of our series suggested nothing new in the direction of treatment. Hydrotheraphy was almost universally practiced and drugs were rarely used and only to relieve definite symptoms. The diet in general was liquid and consisted largely of tinned milk.

PART IV. A STUDY OF RECENT TYPHOID EPIDEMICS IN THE PHILIPPINES.

Many epidemics of typhoid fever have been recorded among troops in the Philippines before this Board began the study of the subject. The outbreaks chronicled below are ones in the investigation of which the Board took a share. The epidemic at San Fernando is of special interest in view of its occurrence in the native population of a town where there was no contact with an American garrison, the adjacent military post of Camp Wallace being occupied by native Scouts.

SAN FERNANDO (UNION) EPIDEMIC.

In July 1910 we learned that an epidemic fever was prevailing in San Fernando and a member of the Board (Captain Bloombergh) was sent to investigate. San Fernando has a population of 16,000 and is located on the coast about 240 kilometers north of Manila. The municipal records show that cholera visited the town in 1908 and is credited with 170 deaths. There are also recorded 34 deaths from intermittent fever, 26 from dysentery and diarrhœa and 9 from menigitis for the same year. In 1909 there are recorded 45 deaths from dysentery and diarrhœa, 12 from intermittent fever and one from meningitis. It is easy to see how typhoid may have been masked by these diagnoses during 1908 and 1909.

Statistics as to the amount of illness prevailing in 1910 were difficult to obtain. A native doctor said there were many cases and a mortality of 50 per cent. An intelligent priest reported much sickness but few deaths. An American teacher stated that out of an average enrollment of 392 children there have been 28 cases of fever with 1 death.

We obtained blood specimens from 25 persons who admitted having had the fever during the preceding 3 months. Two of these were evidently dysentery, and from the remaining 23 we obtained positive Widal reactions in 20. In addition one of the 18 specimens also agglutinated *Bacillus paratyphosus* "B" while all gave negative reactions with *Bacillus paratyphosus* "A". Thirteen other natives who furnished no history of fever gave negative serum reactions with typhoid and paratyphoid bacilli.

Cultures were made from the stools of 4 of the school children ill with a febrile disease, and *Bacillus typhosus* was isolated from two. One of these two patients gave a positive Widal reaction and from the other no blood was obtained.

The sister of this last case had died with a disease of similar clinical nature. In one house visited there had been originally 11 occupants of whom two, both children, had recently died with fever. Eight others were sick at the same time, the mother alone escaping. Of the 8 who had been ill 5 gave positive Widal reactions. These two instances show how the disease may have spread by contact but it must not be forgotten that in San Fernando, as in other Filipino towns, there is excellent opportunity for the spread of the disease by any or all of the recognized methods of infection.

Blood smears from 42 natives, inluding all of the cases recorded above as giving positive typhoid findings, were examined for latent malaria and no organisms were discovered.

FORT MILLS (CORREGIDOR) EPIDEMIC.

Three cases occurred on this island in December, 1910 and January, 1911. The first case was the wife of a white civilian employee. The next two cases were white soldiers. Two other soldiers suffering from continued fever also gave positive Widal reactions combined with negative cultural results from blood and stools. As these two men had received antityphoid vaccination, it is uncertain what the cause of their fever may have been. Several other soldiers with fever gave negative Widal reactions. A fourth case of undoubted typhoid in a native woman occurred in May, 1911.

The source of the infection in the above instances remains undetermined. The sanitary conditions on Corregidor were not favorable at that time because of the crowding and the great amount of construction going on. There was a large American garrison, a smaller force of Filipino troops and several thousand Bilibid convicts and native laborers engaged in work on the island. In addition many of the laborers had their families living in a village on Corregidor. The water supply was unsatisfactory in quantity and quality and the disposal of excreta was for the most part by dry earth closets or even more primitive methods.

CUARTEL DE ESPAÑA (MANILA) EPIDEMIC.

Nine cases occurred in company "M" of the 26th Infantry, all being taken sick within one week. No cases occurred in any of the 7 other companies at the post. The sanitary conditions were very good at this station and were the same for all the companies. The cook for company "M" had a short fever and gave a positive Widal reaction. It was learned that two years before in the United States he had had a fever of a month's duration followed by a relapse also lasting one month. It was thought that he might be a carrier and responsible for the outbreak in his company. However, efforts to demonstrate the bacilli in his stools and urine were unsuccessful. The men attacked were not closely associated with each other in barracks so it seems probable that contact infection was not a factor in this epidemic.

LUDLOW BARRACKS (PARANG) EPIDEMIC.

During the years 1909 and 1910 a very extensive epidemic occurred at this post, there having been 80[3] admissions to hospital in a period of six months beginning October 1, 1909. The official population of the post for the period averaged about 850 persons. Two cases diagnosed as typhoid occurred earlier in the year, July and August, but whether these had any bearing on the susequent epidemic can not be determined. The admissions by months, including civilians, are as follows:

TABLE XVII.—*Admissions for typhoid fever at Ludlow Barracks, Mindanao.*

	1909					1910					Total.
	July.	August.	October.	November.	December.	January.	February.	March.	April.	May.	
Admissions:											
Number	1	1	47	3	4	3	12	[a]11	0	0	[b]82
Per 1,000			52.69	3.45	5.51	3.58	12.15	[a]12.64			
Mean strength of command.			892	868	726	837	988	870			

[a] See footnote 3.

[b] These figures are based on an examination of the post records and the actual clinical histories. They differ from the figures in the office of the chief surgeon, Philippines Division, which showed 4 less cases for October, 1 less for December, 2 less for January, and 2 less for March. The differences are due to certain civilians not being included in the official records.

At this point it may be of interest to note that the records of the post show that there was a small epidemic of enteric fever at Parang in 1902, the admissions having been as follows:

June, 1; August, 1; October, 4; November, 6; December, 1.

At the time of the occurrence of the 1909-1910 epidemic the sanitary conditions at the station, except for the water supply, were good. The post of Ludlow Barracks is located on an elevated, easily drained site, the grounds were well policed and the barracks and quarters were of modern construction. Excellent water closet and bath fixtures had been installed shortly before the outbreak, the outfall being in the ocean about 600 meters from the post. Flies were not common, this being the rule in the Philippines.

At the time of the occurrence of the October cases the garrison had been stationed at Ludlow Barracks for a period greater than the longest recognized incubation period for typhoid fever, even granting that the early cases had been sick a couple of weeks before the disease was discovered.

Distilled water was supplied for drinking, cooking, cleansing of the teeth, and for dish washing and its use enjoined by orders. There was no reason to think this water was contaminated. For flushing of closets and for bathing the supply was obtained from the Nitwan river, a rapid mountain stream draining a country inhabited by Moros and but little known. The water was piped about the post and in the adjacent town of Parang which is largely frequented by the soldiers. That this water at times was used for drinking, dish washing, and cleansing of the teeth can not be gainsaid.(4)

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[3] The Twenty-third Infantry, which formed the garrison at Ludlow Barracks during this epidemic, embarked March 20, 1910, on transport *en route* to San Francisco *via* Manila. At Manila ten suspects were transferred to the Division Hospital. Of these 4 proved to be suffering from typhoid. These should be included in the Parang epidemic, bringing the total number of cases up to 80 in six months. These 4 developing on the transport have been included in the 11 admissions for March in Table XVII.

The Nitwan river is undoubtedly extensively contaminated with fecal matter. (7) It is a custom of the Moros to defecate in running water. That typhoid fever is by no means infrequent among the natives in Mindanao seems certain from the opinions referred to near the beginning of this report and from our own work. The Nitwan water was examined at the times of the two visits made to the post by members of the Board. No typhoid bacilli were isolated from it but its bacterial count was very high and it was extensively infected with the colon bacilus. (5) (6) Examination of the dock water (piped from a spring-fed stream) and of the Alphonso XIII spring, which supplied the swimming pool, showed high bacterial counts and the presence of a few colon bacilli.

The town of Parang is a small village immediately adjacent to the post and depending for its support entirely upon the American garrison. A series of serum reactions with *Bacillus typhosus* made by this Board during the epidemic on 84 of the inhabitants of Parang gave the following results:

TABLE XVIII.—*Widal tests on the inhabitants of Parang, Mindanao.*

Race.	Number examined.	Positive reaction.	Negative reaction.	Sick at time of test.
Chinese	8	1	7	0
Japanese	10	6	4	4
Moros	12	1	11	0
Hindoos	1	0	1	0
Filipinos	53	5	48	1
Total	84	13	71	5

The five reported sick were all suffering from a disease clinically typhoid while two others who gave positive reactions had slight fever but no other symptoms. One of the latter was Japanese and the other a Filipino. A number of these sick persons were Japanese and Filipina prostitutes and their houses were still open to soldiers while the inmates were ill and not in any way isolated.(7) The opportunities to contract infections are obvious.

Major Page, who was Surgeon at Ludlow Barracks during October, November, and December, in a report dated December 22, 1909, expressed the opinion that the epidemic was water-borne, the source of infection being the Nitwan River. He considered that the peculiar grouping of the cases in certain barracks was due to the Nitwan water supply having been first installed in those barracks, shortly before the outbreak. The evidence as to the coincidence of the dates of installing the pipes and the development of the cases in October seemed to us rather conflicting. During the later period of the epidemic, after Major Page's departure, the same peculiar grouping of cases was evident although at that time the water was installed for all the barracks.

The disease continued to occur after Major Page left Ludlow Barracks, flared up in March and April and *then disappeared entirely following the departure of the 23d Infantry and a very complete and thorough disinfection of the* *barracks before the new garrison entered them.* This was carried out under the supervision of Major Clayton who believed the epidemic was spread by contact and not by water.

This Board made two investigations of the epidemic, one in the fall of 1909 and the other in the spring of 1910.(5)(6) From all the evidence at hand several months after the disease had entirely disappeared we concluded:

(1) That the Nitwan river was badly contaminated with human excrement and that very probably some of the cases originated from drinking this impure water at a time when it contained *Bacillus typhosus.* There was no positive evidence to show how the first cases originated.

(2) That the spread of the epidemic was due mainly to contact infection, including in this the probability of cooks or attendants infecting food while handling it in the kitchens and dining rooms. We based this opinion mainly on the following facts: (*a*) The vast majority of the cases for the whole period of six months were peculiarly grouped in certain barracks, one group of adjacent organizations having few or no cases while another group of contiguous buildings had many cases. (*b*) The cases in Parang, where unboiled Nitwan water was probably in very general use for drinking, were all among individuals coming in close personal contact with the troops. (*c*) No cases occurred among the officers and non-commissioned officers living in separate quarters. (*d*) Typhoid disappeared from among the troops at Ludlow Barracks following a change in the garrison and rigid disinfection of the buildings. The period of freedom has now been over a year.[4] (*e*) There was complete failure to suppress the epidemic by means of stringent measures based on the water borne theory.

The dates of admission of the cases to hospital largely supports the contact theory, but too much reliance should not be placed on this point since the clinical records indicate that many men had been sick between one and two weeks before going on sick report.

Efforts were made to locate typhoid bacillus carriers but without success.

A more extended discussion of some features of this epidemic can be found in the references (4), (5), (6), and (7). A "spot map" of the post and the dates of admission are on file in the offices of the Surgeon General of the Army and the chief surgeon, Philippines Division under the heading shown in reference.(6) The clinical aspects of the Parang epidemic are of considerable interest as possibly showing a rather unusually large percentage of extremely mild cases. The diagnosis in all cases was made as a result of a positive Widal reaction (1 to 50 in one hour), nearly all of these serum tests having been performed by members of the Board. None of the patients considered had ever received antityphoid vaccination. In several instances the blood, stool or urine cultures were positive for *Bacillus typhosus.* On many soldiers suffering from slight indispositions the Widal reaction was tried with negative results.

Including the July case and the 4 cases detected after the 23d Infantry left Parang we have clinical records of 81 patients in whom there was a positive Widal reaction. Of these three died, a mortality of 3.7 per cent. One showed at autopsy "congestion and slight ulceration" of Peyer's patches. He apparently died of overwhelming toxæmia about the 17th day of the disease. The second patient died of toxæmia on the 22d day and at autopsy showed the characteristic intestinal lesions of typhoid. The *Bacillus typhosus* was isolated from the heart blood. The third case died of general peritonitis following perforation of the ileum. Autopsy showed enlarged and ulcerated Peyer's patches.

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[4] Eight months after the epidemic ceased one case of typhoid was admitted to the hospital. The patient was a native. Three months later an American civilian was admitted with typhoid fever and two months after this another native, the latter coming from a neighboring *barrio.*

Forty-one cases, constituting 50.6 per cent of the whole, show on the chart a continued fever ranging from sixteen to forty days and averaging 26.4 days. The temperature charts of 33 were classed as typical of typhoid, showing a well marked fastigium which averaged 38°.8 C. in three cases 39°.4 C. in seventeen, 40° C. in twelve, and 40°.5 C. in one.

Diarrhoea was noted in 11, constipation in 21 and regularity of the bowels in 9. Rose spots were recorded in 7 cases and dicrotic pulse in 6. Complications occurred in the shape of multiple peripheral neuritis in one and post-typhoid psychosis, requiring transfer to the United States, in another.

In this group of 41, *Bacillus typhosus* was recovered from the blood of two, the stools of two and the urine of one.

The records of 27 of the cases (constituting 33.3 per cent of the whole) showed temperature curves which resembled the last week or ten days of a typical typhoid chart. In one of these 27 cases *Bacillus typhosus* was isolated from the stool, in another from the urine. It is believed that these patients had been suffering from a mild attack of typhoid fever for periods ranging from five to fifteen days before admission to hospital.

A third group consisting of 10 cases (12.3 per cent of the whole) presented trivial rises of temperature lasting for a few days and bearing no resemblance to the classical typhoid fever chart. All showed clear-cut serum reactions and from the stools of 3 the *Bacillus typhosus* was recovered. There is no reasonable doubt that all were suffering from extremely mild and atypical typhoid fever, or, else were just at the end of an attack which had never caused them to be excused from duty. Their actual appearance on sick report was not due to their own volition but was the result of the general taking of temperatures and admission to hospital of all who showed any degree of fever.

Of the 81 cases considered above 3 were natives and the remainder Americans. The total number considered (81) does not include the 5 cases in the adjacent town of Parang and referred to in Table XIX.

Taken as a whole, the epidemic was characterized by constipation, absence of haemorrhage, very slow pulse, rarely over 100 and often normal, by a large ratio of very mild or ambulatory cases, and by a low mortality, 3.7 per cent. An analysis of the October epidemic was made by Lieutenant Charles T. Dulin and of the whole period of the epidemic by Lieutenant Clarence E. Fronk.

CAMP ELDRIDGE (LOS BAÑOS) EPIDEMIC.

Eleven cases of typhoid fever occurred at this post during July and August, 1909, the garrison consisting of 4 companies of the 7th Infantry. The cases were quite evenly distributed. One patient was a commissioned officer and of the 10 enlisted patients 4 came from "C" company, 3 from "A" company, 2 from "B" company and 1 from "D" company.

The post of Los Baños is situated on an elevated, well-drained site. The earth closet system was in use at the time of the epidemic. The water for drinking purposes was boiled. That for bathing is derived from a stream, the intake being a considerable distance up on Mount Makiling. The possibility of its having been infected can not be excluded. An effort made at this laboratory to find carriers among the company cooks or elsewhere in the command was unsuccessful. The garrison left Fort Wayne, near Detroit, (where typhoid is said to have been common) on April 30, 1909, and arrived at Camp Eldridge on June 6, 1909. The first case of typhoid fever entered hospital July 3, 1909. A study of his chart and of the charts of the next two cases, admitted July 9 and

10 respectively, would indicate that the patients came into the hospital in the first few days of their typhoid course. It appears, therefore, that the infection of these first cases was gained after the troops arrived at Camp Eldridge. As far as could be learned, there was no typhoid fever in the adjacent town of Los Baños except for the case of a soldier who while on furlough had been living at the hotels in Los Baños and developed typhoid there. He subsequently died of the disease in the Division Hospital.(8) The ice and bottled waters produced by a commercial company in Los Baños fell under suspicion, since they were in use among the soldiers. Samples of the bottled products examined by the Bureau of Health in Manila were negative for the bacillus of Eberth.

It was found impossible to locate the origin of the epidemic. Its manner of spread was not definitely determined but was very probably due to flies which were numerous at that time and may have carried the organism from the dry earth closets infected by the first case.

Of the 11 cases reported as typhoid 10 presented temperature charts with febrile periods ranging from twelve to forty-one days and averaging 23.1 days. All but one of these showed a well-marked fastigium. From the blood of 4 the *Bacillus typhosus* was isolated, four others gave positive Widal reactions and in the two remaining cases no Widal reaction was performed but the diagnosis was clear on clinical grounds.

The eleventh case diagnosed as typhoid is of a doubtful nature. The temperature curve is not characteristic and one Widal reaction and one blood culture, both done on the 11th day, were negative.

A large number of mild cases of fever occurring in the command at the time were negative for Widal reaction and blood culture at the Laboratory of the Board for the Study of Tropical Diseases. As dengue was epidemic in the post at the same time as the typhoid outbreak, the number of cases requiring laboratory observations was considerable.

The progress of the disease in this epidemic was characterized by lassitude, headache, constipation, rose spots, enlarged spleen and dicrotic pulse. The charts indicate that constipation existed in 7 patients, regularity in 1, and abnormal frequency of bowel movement in two. Abdominal pain was marked in one case, delirium in another and intestinal hæmorrhage in two. There were no deaths.

CAMP GREGG (BAYAMBANG) EPIDEMIC.

Fifteen cases of typhoid are recorded among the Philippine (native) Scouts at this post between November 26, 1909, and April 5, 1910. Thirteen of these gave positive Widal reactions at the laboratory of the Board. As the other two had an irregular fever and as Widal and stool cultures were negative, we have excluded them from consideration.

This epidemic is of much interest because the entire command at this post is native with the exception of the commissioned officers and a half dozen non-commissioned staff officers.

The post is built on a slight elevation, located in a broad, poorly drained plain. The region is very malarious. The barracks are in rather poor condition. Fæces are disposed of by the dry earth closet system. Flies are not common. Drinking water is distilled. Water for bathing is piped over the post and drawn from the Agno River, a muddy and badly contaminated stream. There is no doubt that the Scouts frequently drink this water in the post and in the town of Bayambang, which adjoins the post. Lieutenant Huber in his report of the epidemic expresses the opinion that typhoid is constantly present in Bayambang and states that he has seen several cases in the families of Scouts living in the town.

The clinical histories of these cases are very meager, but the charts show some points of interest. Two of the cases are classed as typical typhoid, the febrile course being at least twenty days in one and nineteen in the other. There was a well-marked fastigium. One case had a relapse of twelve days' duration and the other a post-typhoid psychosis. Two others might have been diagnosed clinically as very mild typhoid, the febrile period after entry to hospital being between ten and twelve days and the temperature curve not typical. The nine remaining cases showed fever for a week or ten days and may well have been either entirely atypical or the last portion of a typical typhoid chart.

The stools during convalescence were negative in these thirteen cases and cultures from small quantities of blood taken from several in the earlier part of the disease were negative. The specific diagnosis of typhoid rests solely on positive agglutination reactions with *Bacillus typhosus*. We might question the certainty of this method of diagnosis in the atypical cases were it not for the following points: (*a*) Two of the cases gave first a negative reaction followed a few days later by a positive one. (*b*) Many Scouts at the post during the same period who were suffering from mild fevers reacted negatively. (*c*) We have done Widals on several hundred healthy natives and only rarely have obtained a positive reaction.

The writer as representative of the Board at the present time, wishes to acknowledge his obligations to the many medical officers at the Division Hospital and throughout the Philippine Islands who have furnished the materials for the laboratory studies and the clinical records for the analysis, and also to the former members of the Board who did a large part of the laboratory examinations on which this report is based.

PART V. CONCLUSIONS.

1. Typhoid is a widely scattered and common disease in the Philippines; its incidence in Manila is above the average rate for the United States and is exceeded by only a few of the worst American cities; the average admission rate among American soldiers in the Philippines exceeds that for the troops serving in the United States; medical officers from many regions report its frequent occurrence among the Filipinos.
2. The statistics from the Filipino (native) Scouts show a much lower typhoid rate than for white troops, possibly due to failure to diagnose the atypical cases.
3. Widal reactions performed on the blood of 591 healthy Filipinos suggest a comparatively recent attack of typhoid in about 6 per cent of adults, but do not indicate that the disease is prevalent in childhood.
4. Many epidemics have occurred among soldiers in the Philippines and three outbreaks among natives have been studied. Epidemics of great severity among the Filipinos are either rare or unnoticed.
5. The occurrence of the disease in the Philippine Islands is quite evenly distributed throughout the year. The incidence is least in the second quarters.
6. The appearance of the Widal reaction in typhoid fever in the Philippines is not as a rule delayed.

7. Paratyphoid organisms are occasionally isolated in the Philippines.

8. The leucocyte count in typhoid remains normal for whites and is slightly increased for natives. The differential count is normal for both races.

9. The mortality for white troops in the Philippine Islands during the last five years has been no higher than at home. It appears somewhat higher for Filipinos but this may be due to failure to diagnose all the mild cases.

10. More than a third of the cases of enteric fever, whether among Americans or Filipinos, are entirely atypical and can not be diagnosed without laboratory methods.

11. Over one-half of the cases occurring in the Philippine Islands can be diagnosed clinically and differ in no essential particular from typhoid fever as seen at home. This is true for both American and Filipino cases.

12. Much work still needs to be done among the natives to estimate the actual amount of mild and atypical typhoid which is occurring and to determine why extensive and destructive epidemics are not more often seen.

REFERENCES.

(1) Manson, P. Tropical Diseases. London (1907), 311.

(2) Castellani, A. and Chalmers, A. J. Tropical Diseases (1910), 800.

(3) Rogers, L. Fevers in the Tropics (1910), 110 to 148.

(4) Page, Henry. Report of Typhoid Epidemic at Post of Parang. Report made to Surgeon General, U. S. Army, December 22, 1909.

(5) Phalen, J. M. and Kilbourne, E. D. Work of Board for the Study of Tropical Diseases. *Mil. Surgeon* (1910), **27**, 56.

(6) Chamberlain, W. P., Bloombergh, H. D. and Kilbourne, E. D. Report of Board for the Study of Tropical Diseases. The *Mil. Surgeon* (1910), **27**, 529.

(7) Clayton, Jere B. Report as to cause of Epidemic of Typhoid Fever at Ludlow Barracks. Made to Chief Surgeon, Department of Mindanao, April 13, 1910.

(8) Phalen, J. M. and Kilbourne, E. D. Report of Board for the Study of Tropical Diseases. Made to Surgeon General, U. S. Army, September 30, 1909.

(9) Heiser, V. G. Unsolved Health Problems Peculiar to the Philippines. *Phil. Journ. Sci. Sec. B* (1910), **5**, 171.

(10) Jackson, T. W. The Clinical Side of Disease in the Philippine Islands. *Med. Rec.* (1910), **76**, 511.

(11) Nichols, H. J. The Simple and Double Continued Fevers in the Philippines. *Mil. Surgeon* (1908), **22**, 365.

(12) Bruns, E. H. Notes on Epidemic of Typhoid Fever at Iloilo. *Ibid.*, 369.

(13) Nichols, H. J. Medical Survey of Taytay. *Phil. Journ. Sci. Sec. B* (1909), **4**, 281.

(14) Huber, E. G. Report on Typhoid at Camp Gregg. Made to Chief Surgeon, Philippines Division.

(15) LaGarde, L. A. Comments on Epidemic Typhoid with Especial Reference to Contact Infection. *Mil. Surgeon* (1908), **22**, 373.

(16) Report Surgeon General, U. S. Army, Fiscal year 1902, page 56.

(17) Report Surgeon General, U. S. Army, Fiscal year 1903, pages 46, 142 and 151.

(18) Report Surgeon General, U. S. Army, Fiscal Year 1904, page 70.

(19) Osler, W. Modern Medicine. Philadelphia (1907), **2**, 70 to 230.

(20) Hiss, P. H. and Zinsser, H. Text book of Bacteriology (1910), 401.

(21) Strong, R. P. Johns Hopkins Bulletin. Baltimore (1902.)

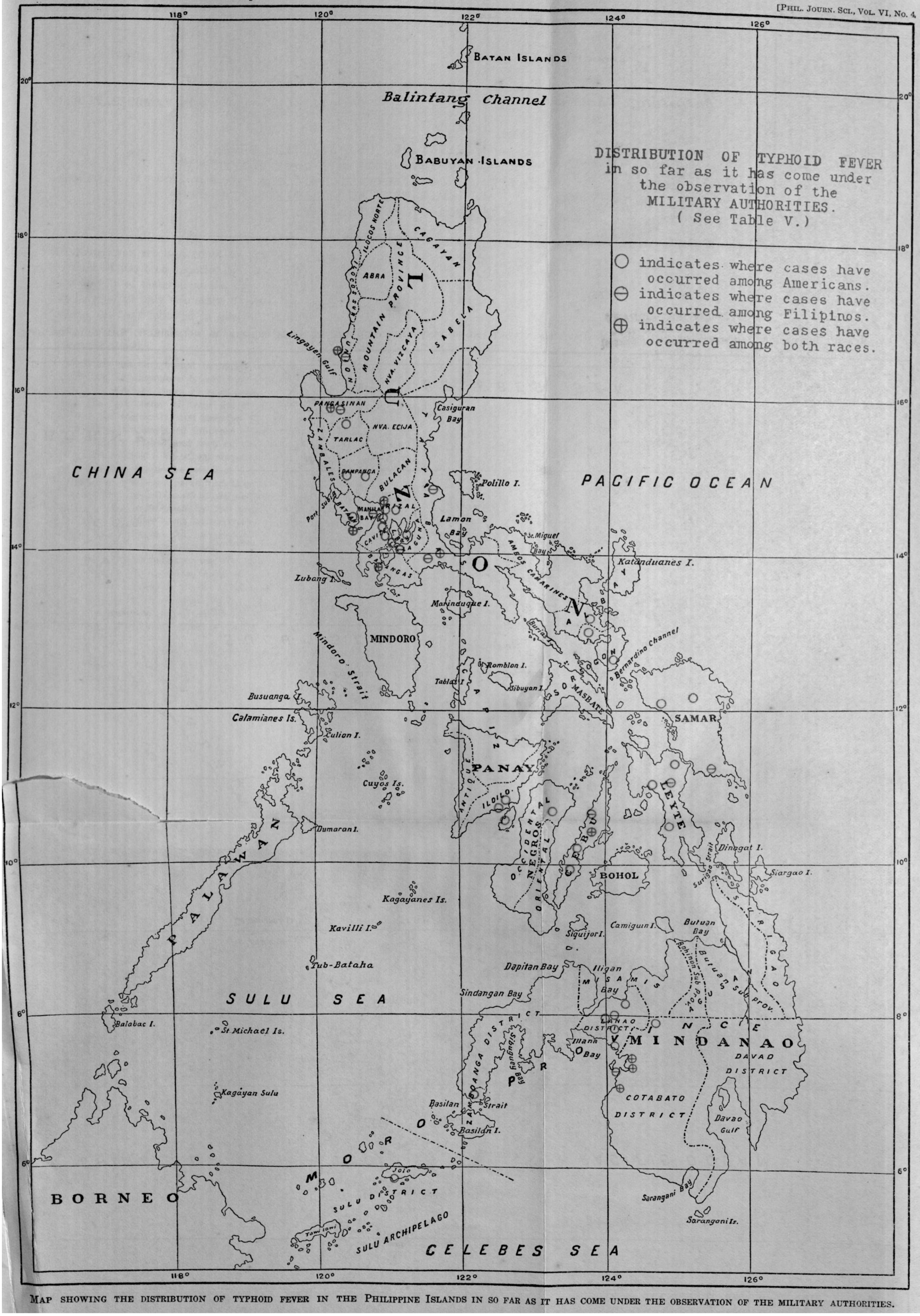
(22) Mortality Statistics. Reports of Census Office. Washington (1908), 32.

(23) Havard V. Military Hygiene (1909), 22.

(24) Editorial, "Manchurian Typhoid" and Brill's Disease. *Med. Rec.* (1911), **79**, 535.

ILLUSTRATION.

Map showing the distribution of typhoid fever in the Philippine Islands in so far as it has come under the observation of the military authorities.



MAP SHOWING THE DISTRIBUTION OF TYPHOID FEVER IN THE PHILIPPINE ISLANDS IN SO FAR AS IT HAS COME UNDER THE OBSERVATION OF THE MILITARY AUTHORITIES.

THE WASSERMAN REACTION IN SYPHILIS, LEPROSY, AND YAWS.[1]

By HORACE D. BLOOMBERGH.[2]

(From the United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands.)

These observations were undertaken with a view of determining if the Wasserman reaction could be obtained in certain tropical diseases in which it has been reported by some authors to be positive. Owing to a scarcity of material at the time the work was being done we were able to examine only two cases of yaws. The methods we employed for making the tests followed in general the original procedure of Wasserman except as regards the antigen, which was prepared from normal guinea-pig heart.

Sheep are not slaughtered in Manila and fresh sheep's blood from different animals can not therefore be obtained. The cells used in all our tests were obtained at different times as needed from the blood of the same sheep. Only during the last series of tests did the suspicion arise that the sheep had been bled too frequently, and therefore the cells were fragile and less resistant than usual to the combined influence of amboceptor and complement. The cells were thoroughly washed and used on the same day as prepared. The dose was 1 cubic centimeter of a 5 per cent suspension in 0.85 per cent salt solution.

The complement was the serum of a guinea pig killed on the day of the test. In this factor we found great variation in concentration. At times the serum was found to be almost devoid of complement. On several occasions we attempted to preserve the complement by the addition of sodium chloride according to the method of Hecht(1) but without good results. The dose of the complement was 0.1 cubic centimeter, or, as employed in actual practice, 1 cubic centimeter of a 1: 9 dilution, in salt solution.

The hæmolytic amboceptor was the inactivated serum of a rabbit which had been immunized by successive doses of well-washed sheep cells. As a rule, we have used the amboceptor in the dilution of 1: 600. Our practice has been to take at least twice the amount which, with 0.1 cubic centimeter of complement, completely hæmolyzed 1 cubic centimeter of a 5 per cent suspension of blood cells in one hour, requiring, however, complete hæmolysis with the quantity used in one-half hour. The invariable dose was one cubic centimeter of the diluted serum. The amboceptor has remained stable for over four months, preserved after inactivation in sealed ampoulles.

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[1] Read, by permission of the Chief Surgeon, Philippines Division, before the Eighth Annual Meeting of the Philippine Islands Medical Association at Manila, P. I., February 22 to 24, 1911.

[2] Major, Medical Corps, United States Army.

The blood serum of the patients as a rule was removed from the veins at the bend of the elbow on the day preceding the test and the serum from the clot was inactivated on the same or following day. It was found later that 0.6 or 0.8 cubic centimeter of serum could be obtained from the blood removed by deep puncture from the finger and collected in small tubes. The serum resulting from the clot was centrifugalized and later placed in similar small tubes, floated on corks in water and inactivated. All inactivation was done at 55° to 56° C. for one-half hour. The serum at first was tried in the two doses of 0.1 and 0.2 cubic centimeter diluted with the necessary quantity of salt solution. Later the dose of 0.2 cubic centimeter was used exclusively.

The antigen was made by grinding guinea-pig heart together with sand in a mortar and adding to this 50 cubic centimeters of 95 per cent alcohol. This was heated to 60° C. for two hours, shaken from time to time, and then filtered. The dose was found to be 0.1 cubic centimeter, twice this amount inhibiting hæmolysis. The antigen has apparently remained a constant factor.

The actual technique of the reaction as carried out by us does not differ from that usually employed. On all occasions on which the reaction was made, a positive syphilitic and a normal serum were used as the main controls of the effective specific action of our reagents. The sera were likewise tested to determine whether they, independently of the antigen, bound the complement or whether they were actively hæmolytic with cells alone, or in the presence of complement. While it was found in actual practice that one hour was a sufficient length of time for the reaction, the final reading was always made at the end of two hours after the addition of the hæmolytic amboceptor and red blood cells.

At the time of our preliminary titration to establish the strength of our amboceptor, we made controls of cells plus complement, amboceptor and antigen, to show that the antigen did not inhibit hæmolysis; and of cells plus complement and of cells plus amboceptor to show that hæmolysis did not occur under these conditions.

It is to be noted first of all that the results reached by us depend on the action of an antigen derived from normal heart and the question of the specificity of action of our antigen comes immediately into question. For our tests we have been unable to obtain syphilitic fœtal organs in the city of Manila, and we may add that the work of others being conducted along lines similar to our work at the present time in Manila, is likewise undertaken with a similar antigen from normal organs. In July, 1910, Wasserman(2) still advocated the use of syphilitic organs in the preparation of the antigen and the good results reached by Dean(3) on the reaction in idiots, referred to by Wasserman in his address, might indicate that in certain lines of research such an antigen is still a necessity. Bayly(4) used an alcoholic extract of rabbit's heart and obtained as good results as with an alcoholic extract of fœtal syphilitic liver. The question is far from being decided but the weight of evidence would seem to indicate that extracts of syphilitic organs are not superior as specific antigens to the extracts of normal organs.

In all, 95 cases have been examined by us. Of these the following 76 gave negative results:

Seventy-six cases showing negative Wasserman reactions.

Number of cases.	Clinical condition.	Number of cases.	Clinical condition.
18	Leprosy.	1	Exophthalmic goitre.
17	Syphilis or suspected syphilis.	1	Otitis media chronic.
9	Syphilophobia.	1	Pemphigus.
5	Dysentery, amœbic, chronic.	1	Rheumatism, muscular.
2	Dysentery, amœbic, acute.	1	Gastritis, alcoholic.
1	Dysentery, bacillary, acute.	1	Neurasthenia and syphilophobia.
2	Rheumatism, articular chronic.		
1	Iritis.	2	Dengue.
1	Malaria, tertian, acute.	1	Ulcer of the leg.
1	Rodent ulcer.	1	Fever, type undetermined.
1	Liver abscess.	1	Osteoma of the tibia.
1	Suspected leper.	6	Healthy Filipinos.

It will be observed that in the above list a certain number of cases are tropical diseases of a protozoal or presumably protozoal nature. It was our desire to work particularly on the reaction as applied to tropical diseases, but we have failed to obtain the material which we most desired.

Apart from the syphilitics enumerated in the table our negative results have not been disappointing, and are entirely in agreement with our ideas of the results of Wasserman reactions properly carried out. Many of the cases were taken from the ward for normal controls, but on account of the habits in general of many of our patients, we habitually used several normal controls at each examination.

Certain cases were examined for purposes of diagnosis, notably the two following:

A case of ulcer which gave a history of a sore of eighteen months' duration on the dorsum of the nose. The first examination made of this patient's serum showed a slight inhibition of hemolysis; subsequent examinations proved that the case was negative. Long continued mercurial treatment had no effect on the condition, and the case was considered to be one of rodent ulcer.

A case of osteoma of the right leg, which gave a history of a chancroid or soft sore on the penis in 1901 and no history of secondaries or any other symptom or sign of syphilitic infection. The man noticed a swelling of the right shin in 1905 and states that occasionally it aches but as a rule he had no trouble in any way due to its presence. The soldier is married and his wife has two healthy children. Until the Wasserman test was used it was thought the tumor was very probably a syphilitic periostitis.

The seventeen cases in which clear luetic histories were obtained, but which gave a negative Wasserman reaction were, to a certain extent, disappointing, although the results were not out of accord with the findings of some other observers, as will be shown in the discussion a little further on. The three following are of interest among the syphilitics who gave negative reactions.

One case was a patient who had severe general rupial lesions and whose serum was examined one week after the administration of Salvarsan ("606"). The case reacted negatively. However, it must be stated that the patient had been for a long time on very vigorous treatment with mercurials. He had not previously been examined with the Wasserman reaction because of our frequent negative results in cases under treatment.

Another, which had been under mercurial treatment only three or four days reacted negatively. This soldier originally had a chancre in the inner canthus of the eye in which one of the Board had demonstrated *Treponema pallidum*. The reaction was made shortly after the appearance of a rash. Whether the absence of the reaction was due to an insufficient liberation of antibodies, or to the treatment received we are of course unable to say.

A third case of more than passing interest was that of right-sided hemiplegia. This undoubtedly was a syphilitic condition occurring as it did in a young man who gave a history of a typical primary lesion, the usual secondaries, an iritis, and finally, almost two years after infection, a hemiplegia with aphasia. This patient had been thoroughly under treatment with mercurials and iodide, particularly for the month or so preceding transfer to the Division Hospital. The Wasserman reaction was negative. That the condition was really syphilitic was demonstrated to our satisfaction by the marvellous improvement which the patient showed after treatment with Salvarsan.

The remaining fourteen syphilitic cases which gave negative reactions had all been under treatment for several months.

A symposium on the theory and workings of the Wasserman reaction was held at the Seventy-eighth Annual Meeting of the British Medical Association, and on this occasion Wasserman(2) made some statements which are pertinent at this point. He said exhaustive investigations have shown that in the majority of recent cases we can cause the disappearance of the reaction through quicksilver treatment. Ehrlich's new arsenic preparation appears to cause in a higher percentage the disappearance of the reaction; whether permanently or not can not yet be decided.

Mulzer,(5) working both with the original Wasserman procedure and with a modified method, has demonstrated that during a mercurial course the serum reaction may change frequently from positive to negative and again to positive at intervals and concludes that it can not be used to determine the sufficiency of a cure, and that for diagnostic purposes it should not be utilized during or immediately after a course of mercurial treatment.

It is useless to repeat the various reports in the literature which show the poor results that are obtained in attempting to secure positive reactions with treated cases of syphilis. Harrison(6) quotes various authors who have proved that heated serum loses its deviating power to a certain extent. He found that after heating for ten minutes at 55° C. the complement for hæmolytic amboceptor was lost and that in *well-treated* latent cases a considerably higher percentage of positive results were obtained by this method than with the same serum heated for thirty or sixty minutes respectively. If this is confirmed, an improved technique in this direction may result for the investigation of the sera of treated cases. We note in his table of results(7) the statement that of 151 cases of secondary syphilis, 136 were positive; 15 were negative, and, in a footnote, he says that 13 of these negative ones had received at least one course of inunctions or injections.

The following is a list of our twenty cases which gave a positive Wasserman reaction.

Twenty cases showing positive Wasserman reactions.

Number of cases.	Clinical condition.	Number of cases.	Clinical condition.
5	Syphilis secondary.	1	Dementia præcox.
7	Syphilis tertiary.	1	Periostitis, chronic.
3	Leprosy.	1	Rheumatism, acute articular (probably syphilitic).
2	Yaws.		

The small percentage of positive results obtained by us in cases of leprosy was referred to briefly in the last quarterly report of the Board. We stated then that 18 out of 21 cases were negative, and that of the three positive, one gave a clear history of syphilis of seven years' duration. The two remaining positive cases passed from observation and we unfortunately were unable to make inquiry from them as to the existence of syphilitic infection or infection with yaws at some previous time.

Wechselmann and Meier(8) have recorded one case of leprosy in which the reaction was positive, using extract of syphilitic liver. The case had originally been considered syphilitic, but later lepra bacilli were demonstrated in the lesions.

Eitner,(9) after having obtained a positive reaction in a case of leprosy with a watery extract of leprous tissue, later secured in a second case a positive reaction using as an antigen an alcoholic extract of normal guinea-pig heart.

Cases referred to frequently in the literature are those of Slatineanu and Danielopolu.(10), (11) Their first report referred to 26 cases of leprosy examined by means of leprous antigen and of these 20 were completely positive and 4 more were reported positive, although the complement fixation was less complete. With alcoholic extract of syphilitic liver their results were different. Of 21 cases examined, 11 were "completely positive," 5 apparently less so, and the remainder negative. At present the general tendency of workers on the Wasserman reaction is to deal with positive and negative reactions only, although occasionally the importance of partial reactions is emphasized. In this connection we might add that almost invariably our results have been distinctly positive or negative and only in rare instances has there been a partial arrest of hæmolysis in negative cases.

Baermann and Wetter,(12) working in Sumatra, found that there was about 7 per cent of manifestly syphilitic individuals present among their laborers and that about 20 per cent of apparently healthy workmen gave a positive Wasserman reaction. Their percentage of positive reactions in leprosy varied between 50 and 65 per cent, the difference depending upon the type of leprous lesions present. Syphilitic antigen was used in their tests.

From our own limited experience we consider it doubtful if a positive Wasserman reaction is to be obtained as a result of infection with the *Bacillus lepræ.* Before attributing a positive reaction in a leper to his leprosy alone it is necessary to consider the possibility not only of syphilitic taint but also of present or antecedent frambœsia. This latter is especially important when working with leprosy in those parts of the Tropics where yaws is prevalent. Recently, we have seen a Filipino leper who also showed typical lesions of yaws.

In yaws we were able to make observations on only two individuals. The serum of one case was forwarded to us by Lieutenant J. R. Barber, Medical Corps, from Corregidor Island, and came from a young Filipino adult in the active stage of the disease. The diagnosis was based on the clinical appearances and on the presence of *Treponema pertenue* in the nodules. The second serum was obtained from a ten-year-old boy who had long been a frequenter of the skin clinic at St. Paul's Hospital in Manila. Both of these sera gave the same strong inhibition of hæmolysis that we obtained from our untreated syphilitic cases. The finding confirms the results of many others who have tried the serum reaction on yaws. It will be of interest to add that seven days after treatment with "606" in the Philippines General Hospital, to which we had transferred the boy and where we obtained the blood a second time through the courtesy of Dr. Donald Gregg, the serum reaction of the second case remained positive. Evidently the time was not sufficiently long for the substances in the blood arising from the activities of the *Treponema pertenue* to disappear. Incidentally, the recovery in this case was remarkably rapid, although the boy had done very poorly on the iodide of potash treatment given him at the clinic over a period of more than a year.

Of the syphilitic cases which gave positive serum reactions few had ever been on mercurial treatment and not one had been recently treated. Four cases gave negative histories of previous syphilitic infection and in two the diagnosis could not have been made without the aid of the Wasserman reaction.

One patient had been transferred to the Division Hospital with the diagnosis of chronic periostitis, cause unknown, and had been on sick report at intervals during the preceding eight months, the total period of disability being more than three months. The diagnosis of syphilis was made by us on the strength of a positive Wasserman reaction. The condition began to improve shortly after the inauguration of mercurial treatment and the soldier was subsequently returned to duty.

A second man, "C. P.," had been transferred to the Division Hospital with the diagnosis of malarial fever after a considerable time on sick report. There were no signs or symptoms in this case other than the presence of fever, and the serum was tested in the expectation of obtaining a negative result. The reaction was positive. The therapeutic test confirmed the diagnosis of syphilis as the febrile condition disappeared promptly on the institution of mercurial treatment.

The third case gave a history of previous syphilis. One thumb, showing rupial ulceration, had been removed on account of the resemblance of the lesion to carcinoma, and the failure of the condition to improve on mercurial treatment. This case gave a good Wasserman reaction in spite of previous treatment with mercury and after an intravenous injection of Salvarsan, the rupial condition, which was then general, disappeared promptly.

Altogether the Board has been pleased with the practical results of the Wasserman reaction and it is a matter of regret that we were unable to make a wider use of it in our study of tropical diseases because of difficulty in securing suitable material at this time.

In conclusion, acknowledgment is made to the various officers of the Division Hospital, particularly Captain Snyder and Lieutenant Shields, from whose wards the majority of our patients were secured, for kindly coöperation and many courtesies extended.

REFERENCES.

(1) Hecht, Hugo. Zur Technik der Seroreaktion bei Syphilis. *Ztschr. f. Immunitätsforsch.* Orig. (1910), **5**, 575.

(2) Wassermann, A. The diagnostic use of the complement fixation method. *Brit. Med. Journ.* (1910), **5**, 1428.

(3) Dean, H. R. An examination of the blood serum of idiots by the Wassermann reaction. *Lancet*, London (1910), **179**, 227.

(4) Bayly, H. W. The practical value of the Wassermann reaction. *Brit. Med. Journ.* (1910), **5**, 1430.

(5) Mulzer, P. Zur Technik und praktischen Verwertung der Wassermannschen Reaktion. *Ztschr. f. Immunitätsforsch.* Orig. (1910), **5**, 247.

(6) Harrison, L. W. The serum diagnosis of syphilis. *Journ. Roy. Army Med. Corps.* (1910), **15**, 39.

(7) Idem. *Ibid.* p. 50.

(8) Wechselmann und Meier. Wassermannsche Reaktion in einem Falle von Lepra. *Deutsche med. Wchnsch.* (1908), **34**, 1340.

(9) Eitner, Ernst. Zur Frage der Anwendung der Komplement-bindungsreaktion auf Lepra. *Wien. klin. Wchnsch.* (1908), **21**, 729.

(10) Slatineanu and Danielopolu. Sur la Présence d'Anticorps spécifiques dans le Sérum des Malades atteints de Lépra. *Compt. rend. Soc. biol.* (1908), **65**, 309.

(11) Idem. Réaction de Fixation avec le Sérum et le Liquide Céphalo-Rachidien des Malades atteints de Lépra, en présence de l'Antigène syphilitique. *Ibid.*, 347.

(12) Baermann and Wetter. Die Wassermann-Neisser-Brucksche Reaktion in den Tropen. *Münchener med. Wchnsch.* (1910), **57**, 2131.

REVIEW.

Practical Bacteriology, Blood Work and Animal Parasitology including Bacteriological Keys, Zoological Tables and Explanatory Clinical Notes. By E. R. Stitt, A. B., Ph. G., M. D., Surgeon, U. S. Navy; Graduate London School of Tropical Medicine; Associate Professor of Medical Zoölogy, Philippine Medical School. * * * Second edition revised and enlarged with 91 illustrations. Cloth. Pp. 345. Price, $1.50. Philadelphia: P. Blakiston's Son & Co., 1910.

The reviewer's prejudice against all pocket manuals is strong. As a rule they contain nothing but what may be had in the larger works, and in the attempt to abridge it becomes a task to know what to omit and what to include. As a result many important facts are omitted, others are only partially stated, if not actually given a wrong setting, and hence the student receives a wrong conception of the actual facts in the case.

While the volume under consideration is no exception to the above criticisms, still it contains some things that the student will not readily find elsewhere. It is more than a compilation of facts from larger works. The author has drawn extensively from his wide experience as a clinical laboratory worker and teacher, both in the temperature zones and in the Tropics, which gives the book a different setting from the ordinary pocket manual, and forms the real worth of the volume. The reviewer would not recommend it as a text-book, but the busy interne in the hospital and the practising physician would find it helpful and suggestive.

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